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BRONCHIAL ASTHMA—ITS ETIOLOGY AND CONTROL*

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MY DISCUSSION of the diagnosis and treatment of bronchial asthma is based on a series of 400 patients, statistics on whom are presented in Table I. No patient was included in this group who had not cooperated with me satisfactorily over a period of at least six months. The conclusions from this study agree with those of a similar study of 234 cases of bronchial asthma presented before the medical section of the A.M.A. in 1925,[1] except in one point—an increased recognition at present of food allergy as a cause of bronchial asthma throughout life. This has been made possible by my recognition of the negative skin reactions and the use of my "elimination diets."[2] It becomes increasingly evident to me that the cause of bronchial asthma is always due to such allergies as will be discussed in this paper. I think that the blame which is still laid at times to nervousness and reflex causes is rarely justified and that undiscovered allergens are practically always operative, offering the challenge of diagnosis and control. That much bronchitis without wheezing is also allergic is important to emphasize.

Most of these patients live on the Pacific Slope especially in the counties around San Francisco Bay and in the Sacramento and San Joaquin Valleys. Many, however, live in the northern and southern counties of California, in Nevada, and a few reside in Oregon, Washington, Idaho, Montana, Arizona and other western states as well as in a few other scattered states over the country. The statistics therefore apply to bronchial asthma as seen in the western states of our country though the conclusions are applicable to the asthmatic problems as observed the world over.

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1. Pollen sensitization in the patients seen in my private clinic occurs more frequently than any other type. This is shown by the occurrence of such reactions in 53 per cent of the patients. Pollen sensitization establishes itself as is the case with other inhalant allergies in most patients after the first year. It is interesting that a larger percentage of the patients in the age group over fifty years gave more positive pollen reactions than in the two younger groups. This emphasizes the importance of routine skin testing of patients of all ages with all types of allergens including pollen. It is interesting that a suggestive history of seasonal pollen allergy occurred in 49 per cent of the patients and that certain patients were found sensitive to pollens in the absence of positive skin tests.

TABLE I

STATISTICAL STUDY OF THE AGES, FAMILY HISTORIES, SKIN REACTIONS, HISTORIES OF SENSITIZATIONS, AND RESULTS IN 400 PATIENTS WITH ASTHMA WHO HAVE COOPERATED FOR A PERIOD OF MORE THAN SIX MONTHS

	157 CASES			194 CASES		49 CASES	TOTAL	PER
	0-5	6-10	11-15	16-35	36-55	55 UP	400 CASES	CENT
	61	64	32	104	90			
Family History	PER CENT			PER CENT		PER CENT	PER CENT	
(1) None	29			50		53	42	42
(2) Unilateral	47			42		41	44	58
(3) Bilateral	24			8		6	14	
Males	60			48		43	52	
Females	40			52		57	48	
Pollen Sensitization								
(1) 1-plus reactions	32		47	26	63	20	27	53
(2) 2- or 3-plus reactions	15			37		18	26	
(3) Suggestive history	41			57		49	49	
Food Sensitization								
(1) 1-plus reactions	31		42	28	35	18	28	36
(2) 2- or 3-plus reactions	11			7		0	8	
(3) Suggestive history	63			59		74	65	
Animal Emanation Sensitization								
(1) 1-plus reactions	38		46	40	51	26	40	49
(2) 2- or 3-plus reactions	8			11		6	9	
(3) Suggestive history	27			26		26	27	
House Dust Sensitization								
(1) 1-plus reactions	45		54	40	55	33	38	49
(2) 2- or 3-plus reactions	9			15		0	11	
(3) Suggestive history	12			36		20	14	
Orris Sensitization								
(1) 1-plus reactions	5		5	9	12	2	7	8
(2) 2- or 3-plus reactions	0			3		2	1	
(3) Suggestive history	0			2		0	1	
Miscellaneous Sensitization								
(1) 1-plus reactions	2		4	6	6	0	4	5
(2) 2- or 3-plus reactions	2			0		0	1	
Bacterial Sensitization	0			2		2	1	
Reactions in Only								
(1) One group	31			24		33	28	
(2) Two groups	25			28		22	26	
(3) Three groups	22			20		10	19	86.2
(4) Four groups	6			14		4	10	
(5) Five groups	0.6			4		0	3	
(6) Six groups				0.5		0	0.2	
History Alone	15			10		35	14	14
Results								
(1) Fair	2			9		8	6	
(2) Good	26			30		45	30	94
(3) Excellent	72			61		47	64	

2. Food sensitization in my opinion is second to pollen as a cause of asthma. My communications during the last few years have emphasized the importance of the history, the occurrence of negative skin reactions and the value of trial diets to determine food allergy. Food sensitization as a cause of bronchial asthma in all ages has been overlooked largely because of the infrequency of satisfactory skin reactions. The occurrence of a suggestive history of food allergy in 65 per cent of these patients as compared with positive skin reactions in 36 per cent illustrates this fact. It is evident that many cases of asthma due to food allergy have been diagnosed as due to bacterial, climatic, or some unknown etiology. It must be mentioned also that many patients who give reactions to foods are not clinically sensitive to those foods. Other patients fail to react to those which actually produce asthma or other allergic manifestations. It is agreed upon by many allergists and especially emphasized by Alexander[3] and myself[14] that skin reactions are not only negative in about 50 per cent of the patients with food allergy but in approximately 10 per cent of the patients with inhalant allergies. As Alexander has pointed out this is due to the tendency for allergy to produce localized phenomena.

3. Animal emanation sensitizations are of marked importance and require careful testing with all types of such allergens. As in other types of allergy, the history is important and there is an occurrence of negative reactions in a few patients.

4. House dust sensitization as discussed by me in 1927[4] continues to be of marked importance in the etiology of bronchial asthma. The routine testing with eight or ten active dust extracts should be done in all patients and the preparation of extracts of dust from the rooms or special furnishings in the patients' living or working environments may be advisable. It is likely that house dust depends for its reactivity on the dusts of the carpets, furnishings, various woods, and clothing and, as Van Leeuwen has emphasized, in certain instances especially in damp humid localities, on fungi.

5. Orris root sensitization is probably the most common of the miscellaneous substances which cause asthma. I reported such allergy in seven cases of hay fever in 1922[5] and found reactions to orris root in 10 per cent of my former series of 234 patients reported in 1925. This checks with the occurrence of such reactions in 8 per cent of my present series of 400 patients. Balyeat[6] has found a greater frequency of orris sensitive patients due, in his opinion, to the freer use of powders in the Southern States as compared with California because of the greater heat and humidity.

6. Miscellaneous sensitizations include those to Pyrethrum, cottonseed and flaxseed especially. In this group are those reactions to allergens whose use has been indicated by the patient's history or occupation. Thus if asthma is not well controlled, testing with furs that are worn, dusts encountered in the occupation of the patient and a consideration of possible drug, dye, or fungus sensitizations[7] are important.

7. Bacterial sensitization as I stated in 1925 probably occurs in a few patients. In this series of 400 patients I felt it was an important cause in only 1 per cent. All other types of allergy should be thoroughly ruled out before

bacterial allergy is blamed for bronchial asthma. As already stated, food allergy especially is at the basis of many cases of asthma which heretofore have been diagnosed as due to bacterial sensitizations. With the use of many autogenous vaccines for the last fourteen years in my treatment of asthma and during the last five years with the use of many separate cultures giving positive skin reactions, I have continued to have the same opinion as I expressed above. The work of Zinsser and others demonstrates that bacterial allergy can certainly occur. In the many cases of bronchial asthma, I think however that most of the improvement which apparently results from vaccine therapy is nonspecific in nature.

ALLERGENS AND METHODS USED IN ROUTINE TESTING

The allergens of all important foods and condiments, of the common pollens of all types to which the patient is exposed, together with those of the important animal emanations, orris root, pyrethrum, cottonseed, flaxseed, 2 or 3 fungi and 8 or 10 active stock house dusts should be used for the routine testing of all patients.

The testing with all allergens should first be carried out with the cutaneous or scratch method. The skin of the back gives good reactions in many patients. The recent studies of Alexander offer an explanation for this fact. The upper arms and forearms can also be used especially for the inhalant allergens. The intradermal tests should be done with the important allergens to which no cutaneous reactions have occurred—usually a maximum of from 30 to 50 tests with the important food and inhalant allergens will suffice. Because of the negative cutaneous reactions, dilutions of 1-200 and later if desired of 1-100 can be used for such intradermal tests with safety. Such intradermal tests should be carefully done, no more than from 0.02 to 0.03 c.c. should be injected and the injection should be made down the outer side of the arms. They should be at least 1 inch apart so that the erythema as well as the wheal can be read. Food allergens for cutaneous testing may be obtained from commercial houses or made in one's own laboratory. Dry pollens are used instead of pollen extracts for the scratch tests. For intradermal testing, food and animal emanation extracts made according to Coca's method, dust extracts prepared by my method referred to above and pollen extracts 1-200 in strength made according to the method of Stier and Hollister[8] are used.

The percentages of patients who gave reactions in from one to six of the groups of allergens listed in my table are of interest. Of the total patients, 28 per cent gave reactions in only one group while 58 per cent gave reactions in from two to six groups. Approximately the same percentages are held for each age group as shown in the detailed statistics.

THE IMPORTANCE OF THOROUGH DIAGNOSTIC STUDIES IN CASES OF ASTHMA

The necessity of a careful and complete physical examination, as well as routine urine and blood analyses, stereoscopic roentgen-ray pictures of the lungs and sinuses must be emphasized. Cardiac and pulmonary diseases must be discovered. Cardiac failure or pleural effusion at times gives symptoms

similar to those of bronchial asthma and as Chevalier Jackson[10] recently pointed out wheezing and difficult breathing may occur from any condition obstructing the bronchial tubes.

SPECIAL COMMENTS ON THE PROBLEM OF BRONCHIAL ASTHMA

1. From the statistics of the 400 case records presented it is evident that multiple sensitizations are the rule. Any combination of pollen, food, animal emanation, dust or miscellaneous sensitizations may occur. It seems that food allergy occurs alone more frequently than does any other type.

2. The occurrence of negative skin reactions referred to above as occurring to all types of allergens and especially to food allergens is again necessary to emphasize. The importance of the patient's history in the determination of possible sensitizations must be stressed.

In cases of bronchial asthma other manifestations due to all types of allergy must be remembered. Food allergy is especially apt to produce such conditions in cases of bronchial asthma in the same way that I have recently reported in the cases of pollen hay fever.[11]

The fact that many gastrointestinal symptoms,[12] migraine and allergic toxemia which I have recently described may be due to food allergy should be fully appreciated. Much localized or generalized abdominal pain, nausea, vomiting, pyrosis, distention, intestinal cramping, diarrhea, constipation and mucous colitis as well as symptoms suggestive of cholecystitis, chronic appendicitis and even intestinal obstruction may be due to food sensitization.[13] In my experience, food allergy is the main cause of migraine and a common cause of ordinary headaches. Canker sores, coated tongue, heavy breath as well as pruritus ani and proctitis are also frequently due to food sensitization. Urticaria, eczema, perennial hay fever, and bladder allergy are often due to the same cause. When such symptoms occur in a patient with bronchial asthma food sensitization should be suspected in the etiology.

TREATMENT

Successful treatment[14] in bronchial asthma demands above all else the patient's cooperation with the physician over a period of many months and in many cases over a period of a year or two. Relief usually occurs as a result of therapy in a few weeks but indicated therapy and diet control must be carried out over a long period of time if results of any permanence are to be obtained. Such prolonged cooperation moreover allows the physician to analyze the causes of any attacks of asthma that may result from the addition of new foods, from the exposure to unsuspected dusts or pollens or from an unintended or deliberate break in the diet. The treatment of bronchial asthma will be discussed under the following headings:

1. Pollen desensitization must be carried out with all important pollens to which the patient reacts. Such therapy at times has to be based on the patient's history when skin reactions are negative. Pollen treatment should be given so that the maximum doses are being administered before the pollinating season begins and these should be repeated throughout the season at weekly intervals. In localities such as my own where grass pollen is in the

air throughout the year,[13] it is necessary to desensitize to such pollens nearly constantly at times for two or more years. Continuous desensitization to fall and tree pollens is also necessary in some patients and preseasonal therapy is important. As I have emphasized in other articles desensitization to the pollens of cultivated flowers is advisable when it is impossible to obviate them.

For the immediate control of symptoms, the use of pollen-free rooms, made so by the use of commercial pollen filters or of a mechanism such as I have installed at the Peralta Hospital by which dehumified, temperature-controlled, as well as filtered air is furnished the patients, may be advisable. The change of location to an area free from the causative pollens such as a seacoast where the wind comes off the sea or in a high elevation may be tried. However with careful pollen therapy repeated every day or two according to Duke's recent recommendations symptoms usually come under fairly rapid control even during the pollinating season.

2. Food allergy must be controlled by the exclusion of foods from the diet, which may be causing the bronchial asthma as indicated by positive skin reactions or the patient's history. Where skin reactions are quite positive, the diet may be prescribed according to these alone. However if relief is not obtained or skin reactions are negative in patients suspected of food allergy the use of my "elimination diets" is strongly recommended.[14]

I feel moreover when the etiology of bronchial asthma is uncertain that the use of diet trial should be routine. This is easily carried out by my standardized elimination diets. When definite food sensitizations are determined by diet trial or skin reactions desensitization may occur as a result of the elimination of such foods over a long period of time or in certain patients by oral or hypodermic desensitization. In my recent book,[14] the many problems of bronchial asthma and especially of food allergy have been discussed.

3. Animal emanation sensitizations as determined by skin reactions or by history require primarily environmental control. By this, I mean the removal from the sleeping and living rooms of all materials containing the animal substances to which skin reactions are positive. If a number of reactions to such emanations occurs, then the removal of all materials of animal origin from the rooms should be carried out. Pillows and mattresses covered with rubberized sheeting, floss pillows, floss mattresses, cotton bedding and washable small rugs should be substituted. The room and all the furniture should be thoroughly cleaned with damp cloths while the windows are shut so that drafts will not blow the surface dusts into the air. Marked sensitizations to common substances such as feathers, wool, horse hair or silk or to emanations of animals which are difficult to evade require specific desensitization. The effect of a brief exposure to an animal emanation causing bronchial asthma is illustrated by the experience of a girl who became sensitive to guinea pig emanations. After being away from such contact for a year she sat in a large room where there were two guinea pigs. After approximately ten minutes asthma began to appear. She immediately left the building and her symptoms were very severe for two days and continued for three more days. This emphasizes the importance of rigid control of the environment of the patient from the point of view of animal emanations to which allergy exists.

4. House dust and occupational dust sensitizations require the same care of the environment as outlined above. As already discussed in this paper, house dust reactions demand strict environmental control. Where a history of sensitization to house dust is obtained and relief of symptoms does not result from such control, dust extracts from various rooms or special furnishings may be prepared. Dusts met with in occupations are apt to produce sensitizations. Desensitization to extracts of such special dusts carefully given over considerable periods of time is often of benefit.

5. Orris root sensitization demands the elimination of all orris root-containing cosmetics, soaps, tooth powders, and shampoos. Some patients are so sensitive that they cannot go into company or theaters or sit in rooms where there are women who have used powders, perfumes or other cosmetics without getting bronchial asthma or nasal congestion. Orris root desensitization in such patients is necessary and of definite benefit.

6. Miscellaneous sensitizations as outlined under etiology demand the eradication of the offending materials from the patient's environment. The desensitization to a specific extract may be necessary. Kapok sensitization must be remembered. A recent patient had marked itching of the eyes which resisted all therapy until a small reaction to kapok was taken into account. It was found that sitting in a room in which there were kapok pillows would result in marked symptoms.

7. Bacterial sensitization as already discussed is rare but should receive attention after all other types of allergy have been eliminated. In a few patients bacterial allergy is present and is relieved by the use of autogenous vaccines or filtrates. Such vaccines can be prepared according to the technic of Thomas and the filtrates according to the technic of Wilmer.[15] Mild bacterial allergies may be secondary to some other allergy such as pollen or food and may be relieved with the control of the main type.

As stated before, the use of stock respiratory vaccines or of autogenous vaccines when an obvious nasal or bronchial infection is present is of much importance. Such vaccines however may depend for their effect on their action as nonspecific protein agents.

SURGERY IN BRONCHIAL ASTHMA

The question of surgery in cases suspected of bacterial infection in the nose and throat is important. I feel that surgery of this type is contraindicated, except in cases of severe and definite infections, until the measures based on all possible allergies as described in this article have been carried out. So much allergic congestion in the nasal passages and sinuses is due to inhalant or food allergies that may or may not be indicated by skin reactions and that may be complicated by mild secondary infections, that I think surgery should only be done on the advice of the allergist after he has brought all existing allergies under as good control as possible.

GENERAL THERAPY

General therapy includes the use of adrenalin, 1-1000 dilution in doses of 5 to 15 minims for relief according to the age of the patient and the severity

of the asthma. Until the therapy based on allergic causes is effective the use of the saturated solution of potassium iodide in doses of 10 to 20 minims two to four times a day is advisable. For persistent asthma the burning of an asthma powder may be allowed though adrenalin and the iodides are more effective and less disagreeable. The use of codein for cough is permissible but not as effective as adrenalin. Morphine should rarely if ever be used to control bronchial asthma. Peptone therapy has been disappointing. Roentgen-ray therapy of the chest or spleen has failed in my hands. Sun therapy and physiotherapy have not been effective or necessary except to improve general health.

THE TREATMENT OF BRONCHIAL ASTHMA WITHOUT SKIN REACTIONS

Certain patients even with the most careful testing give negative reactions. They may or may not give indications in their histories of probable allergies. In these individuals, until relief has been obtained I think that the presence of the common inhalant and food allergies should be assumed and the routine measures outlined below for such possible sensitizations should be carried out.

1. Environmental control as already described should be instituted. In pollen seasons the air of the bedroom should be filtered if possible and empiric pollen therapy with the strongest seasonal pollen extract that fails to give a skin reaction should be given. The patient should live in such an environment until relief occurs and then the effect of exposures to various types of inhalant allergens can gradually be determined. Such procedure would also control possible animal emanation, house dust, orris and other miscellaneous sensitizations.

2. Elimination diets which I have described[14] or some other type of trial diet should be prescribed and taken for seven to fourteen days. If relief occurs foods may be added as I have previously recommended. If no relief occurs modified diets based on possible sensitizations to groups of foods such as cereals, meats, fruits, or vegetables may be advisable or a careful analysis of the effects of the separate foods in the original diets may indicate specific allergies.

3. The routine use of potassium iodide as suggested above is in order. With relief, the dose should be reduced or discontinued to be resumed if symptoms recur.

With these routine measures, the causative inhalant or food allergies can gradually be determined and gratifying relief occurs in many apparently resistant patients. General practitioners who are far removed from free clinics or whose patients cannot afford the expense of travel to the specialist moreover can frequently give such sufferers complete relief by the therapy which has been outlined above for patients who give no skin reactions. Physicians even without the aid of skin tests should try to help such patients with these routine measures. If pollen sensitization is present, the careful use of a pollen antigen obtained through the advice of a specialist or commercial concern should be administered. However it must be stated that complete testing and diagnostic studies at the hands of an allergist are preferable to these procedures. Such specialists are available in large cities and free clinics conducted by them

for the poor are also present. It is realized however that in rural districts or smaller cities, specialists are not available and that many patients cannot leave their local communities because of expense. For these patients, the above suggestions will be effectual in many instances.

RESULTS

The 400 patients included in this series were treated during the last five years since my former article was published in 1925. No patient who had not cooperated with me for at least six months was included. During these years certain patients who have been under my care have not responded to therapy during the first two or three months and have left my care against advice. Such patients have not been considered in this study. The statistics demonstrate the importance of prolonged cooperation in that 94 per cent obtained good or excellent results. Many patients have been entirely free from asthma for upward of two or three years. It is my feeling that some of the 6 per cent who obtained only fair results would have been helped much more if continued cooperation for longer than six months had been obtained.

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THE ACTION OF SODIUM ETHYL PROPYL-METHYL-CARBINYL BARBITURATE (PENTOBARBITAL SODIUM)*†

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IN A PREVIOUS paper, Shonle, Keltch, and Swanson[1] reported the synthesis of a series of dialkyl barbituric acids, including a brief pharmacologic account concerning their hypnotic efficiency and toxicity. It was soon discovered that the sodium salt of ethyl propyl-methyl-carbinyl barbituric acid, a member of the forementioned series, possesses properties common to a few important hypnotics, and a detailed study is therefore deemed advisable. For the sake of brevity, this new compound is provisionally termed sodium iso-amytal, since it is an isomer of sodium amytal. The following is a summary of our pharmacologic data.

1. The hypnotic and anesthetic action of sodium iso-amytal

We have adopted Eddy's scheme[2] to record our results. Thus observations were made on posture, hypnotic effect, heart rate, respiratory rate, analgesia test, rectal temperature, conjunctival reflex, correction of abnormal position, running, righting reflex, knee-jerk, tone of hind-legs, and general condition—first every minute for five minutes after the administration of sodium iso-amytal, next every five minutes during the first half hour, next every fifteen minutes during first three hours, and then every half hour until complete recovery was reached. Rats and dogs were used in our experiments, and different doses of sodium iso-amytal were studied to compare the degree of their hypnosis and anesthesia.

In rats the minimal anesthetic dose lies between 45 and 50 milligrams per kilogram when the drug is given intraperitoneally. The action is prompt, for symptoms of ataxia appear one or two minutes after the administration of sodium iso-amytal. The average duration of anesthesia (judged by the absence of tail reflex with a 500 gram weight) in fifteen rats with a dose of 50 milligrams per kilogram was twenty-seven minutes, and that of hypnosis and stupor three hours and thirty-three minutes.

In dogs it requires 20 milligrams per kilogram by mouth (approximately 25 per cent of the oral minimal lethal dose [M.L.D.] as will be seen below) to produce light sleep, and 40 milligrams per kilogram by mouth (approximately 50 per cent of the oral minimal lethal dose [M.L.D.]) to cause anesthesia. In six dogs, each given by mouth 50 milligrams per kilogram of sodium iso-amytal, dispensed in capsules, the ataxic gait appeared in from five to eight minutes. Response to painful stimulation began to disappear on the average in two hours and forty minutes. The average duration of anesthesia in the six dogs was forty-two minutes, and that of sleep eleven hours and forty minutes. Complete recovery occurred in fifteen hours and

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†Sodium Propyl-Methyl-Carbinyl Barbiturate (Sodium Iso-Amytal) is now known as "Pentobarbital Sodium."

five minutes. During recovery the dogs showed in some cases movements of legs, as if running, later sat up with marked incoordination, and finally stood up with a very unsteady gait.

When sodium iso-amytal is injected intravenously in intact dogs, the effects at once appear. A dose of 12.5 milligrams per kilogram produces light sleep, and a dose of 20 to 25 milligrams per kilogram, anesthesia. Loss of painful sensation with anesthetic doses usually occurs in ten to fifteen minutes. In six dogs each given intravenously 20 milligrams per kilogram, the average duration of anesthesia was determined to be forty-two and one-half minutes, and that of sleep three hours and forty minutes. Residual hypnotic symptoms completely disappeared in eight and one-half hours.

2. Action on circulation and respiration

To determine the effect of sodium iso-amytal on blood pressure, pulse rate, and respiration, three dogs were first anesthetized with ether, and injected with doses varying from 5 to 20 milligrams per kilogram, ether being reduced or sometimes stopped after each injection. The respiration is decreased without exception, both in rate and amplitude. It is not infrequent that the rate is diminished from 80 to 20 per minute, as shown in Fig. 1. If a full anesthetic dose is injected rapidly, the respiration is apt to become irregular.

The change in pulse rate is rather inconstant after an intravenous injection of sodium iso-amytal. This is also true in intact animals to which the drug is given. The rate is, however, more frequently diminished with anesthetic doses, as is illustrated in Fig. 1. In that experiment, the rate decreased from 184 to 158 per minute.

The blood pressure is always lowered by the intravenous administration of sodium iso-amytal. In eleven observations with 5 milligrams per kilogram, the average fall of blood pressure was determined to be 26 millimeters mercury. With larger doses or under deep ether anesthesia, the fall is greater. The lowering of blood pressure usually lasts from one to three minutes, and the recovery is complete. Our studies on a few frogs' hearts with sodium iso-amytal, showed a marked depression both in amplitude and rate. The fall of blood pressure may be chiefly due to cardiac depression.

3. Action on vagus

It has been repeatedly reported by Lieb and Mulinos,[3] Shafer, Underwood and Gaynor,[4] and Garry,[5] that sodium amytal diminishes the vagal response to electrical stimulation. We were interested to find out whether or not sodium iso-amytal has the same action on the vagus nerve. Eleven observations on four etherized dogs with doses varying from 5 to 20 milligrams per kilogram failed to show any inhibition of vagal response to electrical stimulation. A typical experiment is shown in Fig. 1. It thus appears that sodium iso-amytal has little or no action on the vagus nerve.

4. Action on body temperature

With one exception sodium iso-amytal in anesthetic doses was found to lower the body temperature in twelve dogs, six of which received the drug

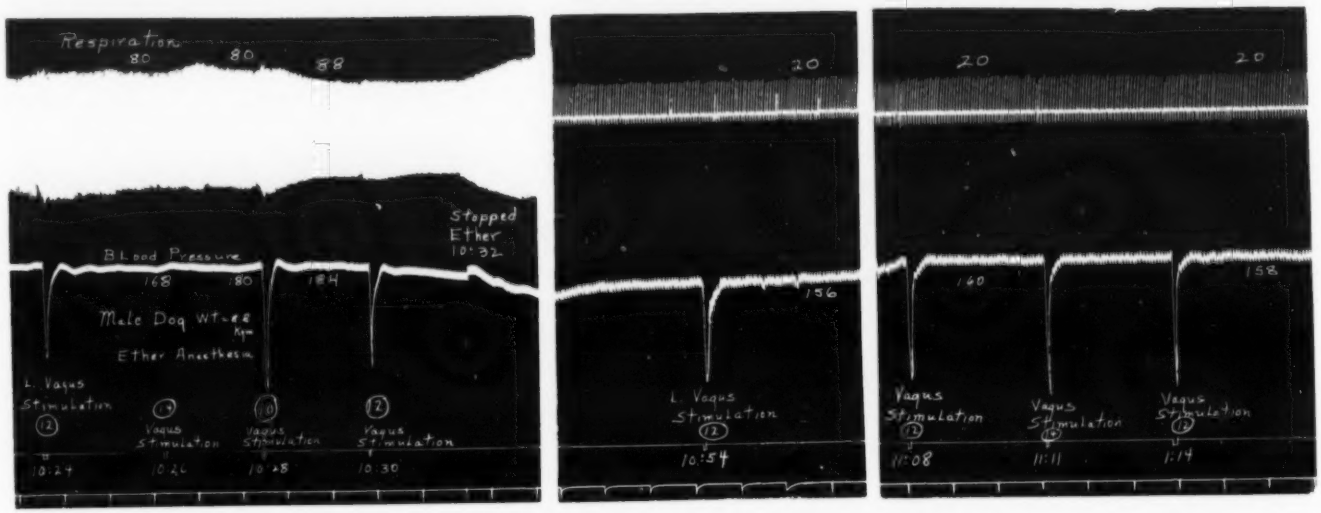


Fig. 1.—Showing the action of sodium iso-amytal on respiration, pulse rate, and the vagus nerve.

Dog, male, weighing 8.8 kg. The blood pressure and respiration were recorded. Ether was used to operate and attach manometer and respiration tambour. The left vagus was cut. Electrical stimulation of the peripheral end of the cut vagus was produced by an inductorium. The minimal amount of electrical stimulation used to produce a distinct inhibition of the heart and a drop in blood pressure was determined as shown in the tracing. The strengths of electrical stimulation under ether were: 12 points, 14 points, 10 points, and 12 points, respectively. The least amount of current required to produce a distinct inhibition of the heart and drop in blood pressure was taken to be 12 points. At 10:36 sodium iso-amytal in 5 per cent solution (25 mg. per kg) was injected intravenously at the rate of 1 c.c. per minute. At 10:54 electrical stimulation of the vagus showed the same degree of inhibition after the injection of the compound as it did with ether. Later at 11:08, 11:11, and 11:14 electrical stimulations were made.

This tracing shows that sodium iso-amytal has no effect on the vagus with anesthetic doses. The respiration, however, dropped from 80-88 down to 20 per minute and pulse rate decreased from 184 to 156.

by mouth, while the remaining six received the drug by intravenous injection. The fall of temperature after oral administration varied from 0.4° Fahrenheit to 4.8° Fahrenheit, the average being 2.6° Fahrenheit. With the intravenous administration, the fall in five animals varied from 0.4° Fahrenheit to 2.1° Fahrenheit, average being 1.25° Fahrenheit. Only one dog had a rise of 2° Fahrenheit of body temperature. The results are summarized in Table I.

TABLE I

ACTION OF SODIUM ISO-AMYTAL ON BODY TEMPERATURE IN DOGS

DOG NUMBER	SODIUM ISO-AMYTAL MG. PER KG.	METHOD OF ADMINISTRATION	MAXIMUM CHANGE IN BODY TEMPERATURE IN 0° F.	AVERAGE
1	35	Oral	−4.8	
2	35	Oral	−1.7	
3	35	Oral	−3.8	
4	35	Oral	−0.4	−2.50° F.
5	35	Oral	−3.6	
6	35	Oral	−1.2	
7	20	Intravenous	−2.1	
8	20	Intravenous	−1.4	
9	20	Intravenous	−1.6	
10	20	Intravenous	−0.8	−1.25° F.
11	20	Intravenous	−0.4	
12	20	Intravenous	+2.0	

5. TOXICITY

The minimal lethal dose (M.L.D.) of sodium iso-amytal was determined intraperitoneally in rats, and both orally and intravenously in dogs, using dose intervals of 5 milligrams per kilogram. The minimal lethal dose (M.L.D.) was taken to be that dose which proved fatal to at least three out of five animals.

As shown in Tables II to IV, the minimal lethal dose (M.L.D.) in white rats by intraperitoneal injection is 120 milligrams per kilograms, and that in dogs by oral administration 85 milligrams per kilogram. The minimal lethal dose (M.L.D.) in dogs by intravenous injection is 50 milligrams per kilogram if the drug in 5 per cent solution is injected at the rate of 1 cubic centimeter per minute. Very rapid injection will show a smaller figure, that is, greater toxicity.

By using this method of determining the minimum lethal dose (M.L.D.) and also the minimal anesthetic dose (M.A.D.), the amount that is sufficient

TABLE II

THE TOXICITY OF SODIUM ISO-AMYTAL IN RATS BY THE INTRAPERITONEAL INJECTION
(Solution used 5 per cent)

NUMBER OF RATS	DOSE MG. PER KG.	NUMBER DIED	NUMBER SURVIVED	M. L. D. MG. PER KG.
5	90	1	4	
5	100	1	4	
5	110	2	3	
5	120	3	2	120
5	130	5	0	

TABLE III

THE TOXICITY OF SODIUM ISO-AMYTAL IN DOGS BY THE ORAL ADMINISTRATION

NUMBER OF DOGS	DOSE PER KG.	NUMBER DIED	NUMBER SURVIVED	M. L. D. MG. PER KG.
10	20 mg.	0	10	
10	25 mg.	0	10	
6	30 mg.	0	6	
6	35 mg.	0	6	
5	40 mg.	0	5	
3	45 mg.	0	3	
4	50 mg.	0	4	
3	55 mg.	0	3	
5	60 mg.	0	5	
5	65 mg.	1	4	
5	70 mg.	1	4	
5	75 mg.	1	4	
5	80 mg.	2	3	
5	85 mg.	5	0	85
5	90 mg.	3	2	
3	95 mg.	3	0	
3	100 mg.	3	0	

TABLE IV

THE TOXICITY OF SODIUM ISO-AMYTAL IN DOGS BY INTRAVENOUS INJECTION
(Solution Used 5 Per Cent, Rate of Injection 1 c.c. Per Minute)

NUMBER OF DOGS	DOSE PER KG.	NUMBER DIED	NUMBER SURVIVED	M. L. D. MG. PER KG.
20	20 mg.	0	20	
5	30 mg.	0	5	
10	40 mg.	4	6	
5	45 mg.	1	4	
5	50 mg.	3	2	50
5	55 mg.	4	1	

TABLE V

DETERMINATION OF THERAPEUTIC RATIO (M.L.D./M.A.D.) IN WHITE RATS BY INTRAPERITONEAL INJECTION

NO. OF SERIES	THERAPEUTIC RATIO
1	2.00
2	2.17
3	2.65
4	3.20
5	3.00
6	2.25
7	2.60
8	2.57
9	2.28
10	2.25
11	2.66
Average	2.51

to cause the absence of tail response to the pressure of a 500-gram weight, we determined the therapeutic ratio, $\frac{\text{M.L.D.}}{\text{M.A.D.}}$. About thirty-five to forty-five animals were necessary for each determination. Out of eleven separate sets of results obtained at different times, the therapeutic ratio in rats by intraperitoneal injection was found to be 2.51, as shown in Table V.

Death from sodium iso-amytal is most probably due to respiratory failure, because respiration often ceases before the cardiac arrest. This is more apparent if the drug is given intravenously.

6. REPEATED ADMINISTRATION AND ELIMINATION OF SODIUM ISO-AMYTAL

To test whether or not there is any cumulation or development of tolerance, three dogs were given intravenously anesthetic doses, every forty-eight hours for eight days. As shown in Table VI, anesthesia and hypnosis ap-

TABLE VI

REPEATED INTRAVENOUS ADMINISTRATION OF ANESTHETIC DOSES OF SODIUM ISO-AMYTAL IN DOGS

(Five Per Cent Solution Used)

DOG NO.	DATE	SODIUM ISO-AMYTAL MG. PER KG.	PERIOD OF ANESTHESIA IN MINUTES	RESULTS		OTHER REMARKS
				DURATION OF SLEEP IN HOURS	DURATION OF ACTION IN HOURS	
151	12: 9	25	75	6	9	
	12:11	25	90	5	8½	
	12:13	25	60	4	7	
	12:15	25	45	4	7	
	12:17	50				Died
152	12: 9	25	60	6½	10	
	12:11	25	105	7	12	
	12:13	25	60	4	7½	
	12:15	25	60	4½	7½	
	12:17	50				Died
153	12: 9	25	45	4	8½	
	12:11	25	75	4	8½	
	12:13	25	75	6	9	
	12:15	25	50	5	7¾	
	12:18	50				Survived

peared after each injection. Although there was a tendency toward decrease in the duration of action in the first two animals, the results from the third showed the reverse. The difference in duration of action probably can be ascribed to the difference in response of the same animals on different days, a condition well known among physiologists. In any event, there is little or no evidence that sodium iso-amytal produces tolerance. This is further substantiated by the fact that a minimal lethal dose (M.L.D.) (50 milligrams per kilogram) killed two out of three treated animals.

The exact mode of elimination of sodium iso-amytal is not yet known. Examination of urine collected from dogs given repeated doses of sodium iso-amytal revealed no trace of the drug administered. Considering the data presented in Table VI, it can be safely stated that dogs can destroy in their bodies at least 25 milligrams per kilogram of sodium iso-amytal within forty-eight hours, which is 50 per cent of the minimal lethal dose (M.L.D.) determined intravenously, as previously shown (Table IV).

7. DETOXICATION OF ACUTE COCAINE POISONING

Following the work of Knoefel, Herwick and Loevenhart[6] and Isenberger,[7] who showed that sodium amytal protects the animals from poison-

ing by local anesthetics, we attempted to determine the toxicity of cocaine in the presence of sodium iso-amytal. The maximal tolerated dose of the depressant drug by intravenous injection in rabbits was found to be 30 milligrams per kilogram, and the minimal lethal dose (M.L.D.) of cocaine hydrochloride given alone subcutaneously in rabbits was taken to be 100 milligrams per kilogram (see Knoefel, Herwick and Loevenhart[6]). To ascertain any prophylaxis against poisoning by the local anesthetic, 30 milligrams per kilogram of sodium iso-amytal (5 per cent solution) were injected intravenously, immediately followed subcutaneously by fatal doses of cocaine hydrochloride (10 per cent solution). Our results (Table VII) show that only one animal died from three minimal lethal doses (M.L.D.) of cocaine, while the others survived. One animal recovered from five minimal lethal doses (M.L.D.) of cocaine. These data appear to indicate clearly that so-

TABLE VIII

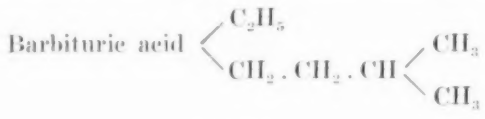
PROPHYLAXIS OF ACUTE COCAINE POISONING IN RABBITS BY INTRAVENOUS INJECTION OF SODIUM ISO-AMYTAL

M.L.D. OF COCAINE HYDROCHLORIDE WITHOUT PROPHYLAXIS MG. PER KG.	COCAINE PLUS 30 MG. PER KG. OF SODIUM ISO-AMYTAL		
	DOSE OF COCAINE HYDROCHLORIDE MG. PER KG.	NUMBER OF RABBITS USED	RESULT
100	200	1	Survived
	300	1	Died next morning
	400	1	Survived
	500	1	Survived

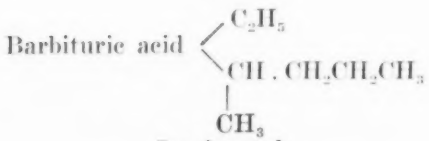
dium iso-amytal protects the rabbits from acute cocaine poisoning, but they are of course too few to permit us to state the exact extent of protection.

DISCUSSION

During the past few years, we have been studying the effects of sodium amytal. The present compound, sodium iso-amytal, is an isomer of the latter. As shown in the accompanying formulas, the only difference between the two compounds lies in the alkyl side chain, though of an equal num-



Amytal



Iso-Amytal

ber of C-atoms. In the amytal molecule a methyl group is attached to the γ-C-atom of the side chain, while in the iso-amytal molecule the methyl group is linked to the α-C-atom. The position of this methyl group apparently has

a great influence on the physiologic activity. By comparing our results on sodium iso-amytal presented here with those on sodium amytal[8] (in part unpublished), it at once becomes obvious that sodium iso-amytal is approximately twice as potent but twice as toxic as sodium amytal. Both drugs have about the same therapeutic ratio. The margin of safety is therefore the same. In proportionate dosage (with the anesthetic dose of each drug, for example), both sodium iso-amytal and sodium amytal have the same onset of action and duration of anesthesia. There is no difference in intensity of action. Both drugs decrease the body temperature, depress respiration, and lower blood pressure.

There are, however, certain differences between the two drugs. In dogs, sodium iso-amytal produces shorter sleep and brings about an earlier recovery than sodium amytal.

Another point of difference, which is of unusual interest, is that sodium iso-amytal does not influence the vagal response to electrical stimulation, while sodium amytal inhibits it.[3, 4, 5]

SUMMARY

1. Sodium ethyl propyl-methyl-carbinyl barbiturate (sodium iso-amytal) is an efficient hypnotic and anesthetic drug in rats and dogs.

2. It lowers blood pressure when injected intravenously, and in anesthetic doses depresses respiration almost by any method of administration. It also diminishes the body temperature.

3. The minimal lethal dose (M.L.D.) in rats by intraperitoneal injection is 120, that in dogs by mouth 85, and that in dogs by intravenous administration 50 milligrams per kilogram.

4. No evidence is obtained that repeated injections of anesthetic doses every forty-eight hours in dogs (five in all) produce any tolerance to the drug.

5. The drug does not inhibit the vagal response to electrical stimulation.

6. It appears to protect rabbits from acute cocaine poisoning.

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A CLINICAL COMPARISON OF ACETYL-SALICYLIC ACID ANALGESIA WITH AND WITHOUT MAGNESIUM OXIDE*†‡

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THE absorption of magnesium following the oral administration of magnesium oxide or chloride is greater than has generally been supposed. For example Winter and Richey[1] have found the human serum magnesium increased in many cases by 0.5 mg. per cent after ingestion of MgO. Furthermore the increase was usually greater in the presence of acetyl-salicylic acid.

These facts together with that of the sedative action of magnesium upon the central nervous system, have stimulated the study of the combined action of magnesium preparations with certain analgesic and hypnotic drugs (amidopyrine, salicylates, cinchophen, phenacetin and barbital). In particular, Barbour and Winter[2] found salicylate action augmented by magnesium chloride given subcutaneously with sodium salicylate in rabbits, and aspirin action increased by magnesium oxide when the two were given orally in dogs. In both these cases the criterion was antipyresis. The same authors found a combination of magnesium chloride and sodium salicylate less toxic for mice than predicted from the separate toxicities of the drugs. For these reasons it seems important to establish whether or not magnesium can be clinically combined to advantage with aspirin. The use of magnesium oxide in particular would be expected to afford protection against gastric irritation.

In the following study acetyl-salicylic acid given alone was compared with the same drug given with magnesium oxide, in the same patients. The drugs, in the form of tablets, were taken with large amounts of water. The amounts and composition of the two drugs administered are shown in Table I.

Patients whose pain was more or less constant were selected so that conditions would be as nearly similar as possible on different days. Furthermore, great care was exercised in excluding those who because of lack of intelligence or other reasons, did not cooperate satisfactorily. Furthermore, where there was any doubt as to whether the relief was real or imaginary, control tests were made with sodium bicarbonate tablets. All patients claiming to be relieved by the latter therapy were discarded as unsuitable subjects. The types of pain found most suitable for study were those accompanying several varieties of arthritis and neuritis as well as headaches of known and unknown origin. Among these conditions the headaches responded better to both drugs than did the other types of pain. The drugs were alternated, one being given for one or more days, three to four times per day, and then discontinued. If

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†The combination was given in the form of "Magnespirin."

‡In cooperation with the Pharmaceutical Division of the Calco Chemical Company, Bound Brook, New Jersey.

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the pain continued or recurred in apparently the same degree as before medication, the other drug was given at the same intervals for a like period.

Acetyl-salicylic acid was given to 45 patients, 98 times, and the mixture with magnesium oxide was given to the same 45 patients, 91 times. In some cases one drug was given first, in other cases the other. The results are recorded in the following term: no relief or very slight relief; moderate relief; very much relief; complete relief. The frequency of several degrees of relief is expressed in percentage in Table I.

TABLE I

FREQUENCY DISTRIBUTION OF VARIOUS DEGREES OF RELIEF FROM PAIN IN 45 PATIENTS TREATED WITH ACETYL-SALICYLIC ACID AND IN THE SAME PATIENTS TREATED WITH ACETYL-SALICYLIC ACID PLUS MAGNESIUM OXIDE

DOSE	ACETYL-SALICYLIC ACID			ACETYL-SALICYLIC ACID PLUS MAGNESIUM OXIDE			
	10 GRAINS	15 GRAINS	20 GRAINS	6⅔ GRAINS OF EACH	10 GRAINS OF EACH	13⅓ GRAINS OF EACH	20 GRAINS OF EACH
Number of courses of treatment	76	22	11	43	48	14	9
No relief or very slight relief	48%	60%	36%	37%	50%	43%	56%
Moderate relief	25%	23%	36%	37%	12%	50%	22%
Very much relief	24%	18%	27%	19%	33%	7%	0
Complete relief	3%	0	0	7%	4%	0	22%

Such results are not easily evaluated but the following points may be noted:

While complete relief was always the first objective, it is evident that this was obtained much more frequently following the use of the magnesium combination, which gave seven cases of complete relief against two with acetylsalicylic acid alone. It may be added further that six of the former group fell short of complete relief with acetylsalicylic acid while the combination failed in one of the latter group.

Evidently six and two-thirds grains of aspirin given with the same amount of magnesium oxide was more frequently effective and yielded complete relief more often than did ten or even fifteen grains of aspirin alone. In other words, it would appear that magnesium oxide can, often at least, reduce the amount of aspirin necessary to produce a certain effect.

It is evident that the higher doses of both drugs failed more often to give significant relief than the minimal doses just mentioned. This is explained simply by the fact that larger doses were only given in the more refractory cases. For this reason, the first column under each drug probably affords the best criterion for comparison of relative efficiency.

Abdominal discomfort was experienced by seven patients after taking acetyl-salicylic acid. Among these was the only patient in the entire series complaining of abdominal discomfort after the mixture; in this case the existence of such discomfort was in doubt after thorough interrogation.

CONCLUSIONS

Clinical comparison between acetyl-salicylic acid and a combination of acetyl-salicylic acid and magnesium oxide was made in 43 patients given both.

The smallest dose of the combination tested gave significant relief more frequently than did larger amounts of aspirin alone. In the whole series complete relief was afforded more than twice as often by the combination as by the single drug. It appears true for many individuals that the addition of magnesium oxide reduces the amount of acetyl-salicylic acid necessary to produce a given degree of analgesia.

Symptoms of gastric irritation following the oral administration of acetyl-salicylic acid are practically eliminated by the addition of magnesium oxide.

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THE INFLUENCE OF ETHYL ALCOHOL UPON THE GASTRIC ABSORPTION OF PHENOL IN RABBITS*

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ALTHOUGH the value of ethyl alcohol in phenol poisoning has been questioned, most lists of antidotes still give it a prominent place. In surgery the excess phenol from cauterization is commonly removed with alcohol. Wilbert[1] in a review and study of cases concluded that the beneficial effects of alcohol in phenol poisoning had been greatly exaggerated. Macht[2] in an experimental study concluded that alcohol was harmful if given as a lavage after phenol ingestion, but might be beneficial if given before phenol ingestion. Peterson, Haines, and Webster[3] have condemned the use of alcohol as a lavage in phenol poisoning.

Our interest in this problem arose when we were attempting to produce experimental achylia in dogs by swabbing the mucosa with phenol through gastric fistulae followed by alcohol to remove the excess phenol. We were

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impressed with the increase in toxic manifestations following the use of alcohol. Carbon dioxide inhalations produced a prompt recovery suggesting that the toxicity was due to alcohol poisoning. However, the development of blood phenol methods first by Pelkan and later by Rakestraw[4] made it possible to decide more accurately whether or not the increased toxicity was due to (1) an increased absorption of phenol, (2) a superadded alcohol poisoning, or (3) both. A comparison of the blood phenol levels in two groups of animals, one having received phenol alone, and another a mixture of phenol and alcohol should furnish the answer. Although the present blood phenol methods are not entirely satisfactory as regards specificity, yet by using healthy fasting animals defects inherent in the method should not affect the comparative value of the data. Macht's careful work was done in 1915 and his conclusions were of necessity based upon the appearance time of convulsions and time of death in his animals. The addition of blood phenol levels should complement his data.

METHOD

Nine animals received phenol orally and five received a mixture of phenol plus an equal amount of absolute alcohol. The animals were large, healthy rabbits, and were fasted twenty-four hours before receiving the solution orally through a rubber catheter. Attempts to apply phenol directly to the stomach mucosa, duodenum, or into closed intestinal loops after trials, did not appear superior to the use of the oral method. Blood samples were obtained by heart puncture and the free and conjugated phenol determined by Rakestraw's method. The glassware was calibrated by the Bureau of Standards

TABLE I

	MG. PHENOL PER 100 C.C. OF BLOOD AFTER INGESTION						DEATH	CONVULSIONS
	0 MIN.	10-20 MIN.	21-30 MIN.	31-40 MIN.	41-60 MIN.	61-80 MIN.	MIN.	MIN.
Phenol group								
P-1 5 c.c.			11				45	
P-2 5 c.c.		15	11	8.5	19		50	
P-3 5 c.c.		14	11				30	5
P-8 5 c.c.	3.8	19	18				21	
P-9 3 c.c.	5.3	11	7.4		29		53	40
					26			
P-11 3 c.c.	3.6	31	31	32	33	34	85	2
P-12 3 c.c.	3.8	18	21	30	35		57	2
P-13 3 c.c.	6.2	18	17				21	
		(18)						
P-14 2 c.c.	5.9	24	27	28	29		86*	5
	5.8	18	19	27	29	34	45	
Phenol plus alcohol group								
P-4 5 c.c. each		17					20	5
P-5 5 c.c. each		31		26	25	31†		
P-6 5 c.c. each		26	28			34‡	123	
P-7 5 c.c. each		25	34		44		50	3
P-15 3 c.c. each	3.7	28	32	32		34	78	
	3.7	25	31	29	35	33	56	

*Killed
†83 minutes
‡123 minutes
Figures in ( ) indicate extra determinations during same time interval.

or checked in our laboratory and the standards and reagents were checked frequently. When a sufficient sample could be obtained the analyses were duplicated. The appearance time of convulsions was noted as well as the time of death. At the completion of the experiment, except for one or two instances, each animal was autopsied and the experiment rejected if pericardial hemorrhage had occurred or if the phenol had not been introduced into the stomach.

The results are recorded in Tables I and II.

TABLE II

CONJUGATED PHENOLS

	MG. CONJUGATED PHENOL PER 100 C.C. OF BLOOD AFTER INGESTION					
	0 MIN.	10-20 MIN.	21-30 MIN.	31-40 MIN.	41-60 MIN.	61-80 MIN.
Phenol group						
P-11	1.6					
P-12	3.1	1.5	1.5	2.8	2.5	
P-13	1.8	1.5	2.0			
P-14		3.3	3.8	5.5	5.7	
Phenol plus alcohol group						
P-15	1.6	1.2	1.2	0.9		3.6

CONCLUSIONS AND SUMMARY

1. The appearance time of convulsions and the time of death in nine rabbits receiving phenol alone was approximately the same as a group of five receiving a mixture of phenol and alcohol.

2. The group receiving the phenol and alcohol mixture showed a somewhat more rapid rise in free blood phenol but the final levels reached before death in the two groups were essentially the same.

3. The rise in conjugated phenols was very slight.

4. The effect of alcohol on the gastric absorption of phenol is slight and the *apparent* increase in toxicity is probably due to a superadded alcohol poisoning.

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COMPARISON OF METHODS USED IN THE STUDY OF ANAPHYLAXIS*†

By Bret Ratner, M.D., and Helen Lee Gruehl, B.A., New York, N. Y.

SINCE Richet[1] in 1902 described the phenomenon of anaphylaxis, many methods have been devised for its study. A voluminous literature has developed, but as Wells[2] so aptly states much unnecessary work might have been avoided and less confusion would exist if the criteria for anaphylaxis were better understood and observed.

Certain standards are accepted for the solution to be used, the incubation period necessary for the establishment of sensitization, and the symptoms typical of anaphylactic intoxication. But, there appears to be less unity of opinion as to the best method for clearly and regularly demonstrating the degree of hypersensitiveness.

The early investigators used the subcutaneous route for both sensitizing and shock injections. But this is generally discarded because of its inaccuracy, and the intraperitoneal route adopted in its stead. The intracardiac route has been rejected because of the difficulty of injecting solutions into the heart and the possibility of damage to the myocardium, hemorrhage, and nonspecific shock. The intrathecal route, introduced by Besredka[3] is not generally used because it is nonspecific and because the neurogenic theory for anaphylaxis is not supported by adequate evidence. The methods most commonly used to determine hypersensitiveness in animals are injections via the intraperitoneal or intravenous routes, and the Schultz-Dale uterine strip method.

When we recently devised the method of producing anaphylactic intoxication through the inhalation of a dry antigenic dust,[4] we had to prove that the reaction, in the form of dyspnea, was evidence of anaphylactic shock. We, therefore, injected dust-sensitized animals intravenously with an extract of the same antigenic dust. They died in typical shock, thus proving our contention that the respiratory embarrassment, which so closely resembles asthmatic symptoms in the human being, was truly a symptom of anaphylactic intoxication. Similarly, in other experiments we found that in instances where the intraperitoneal route resulted in a negative or mild reaction more definite symptoms were elicited by a subsequent intravenous injection.

The question arose, therefore, as to which of the methods would most uniformly elicit symptoms of anaphylactic intoxication in the guinea pig. A series of comparative studies was undertaken to discover the relative merits of the other shock methods as compared with the intravenous.

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EXPERIMENTAL

Animals were sensitized by intraperitoneal or intravenous injection, by inhalation, or by passive transfer. The usual incubation period of two weeks or longer was observed.

Solutions Used.—For these experiments 2 solutions were used. The first was normal horse serum (HS). The second solution was an extract of horse dander (DE) prepared by a method previously described.[4] This solution was always freshly made on the day it was used. In the early part of our work the P_H of the solution was adjusted, but we found that this adjustment was of no particular value as the P_H of guinea pig's blood varies considerably.[4]

No solution was used either for injection or for the uterine experiment unless its toxicity was determined. Each solution was Berkefelded so that it

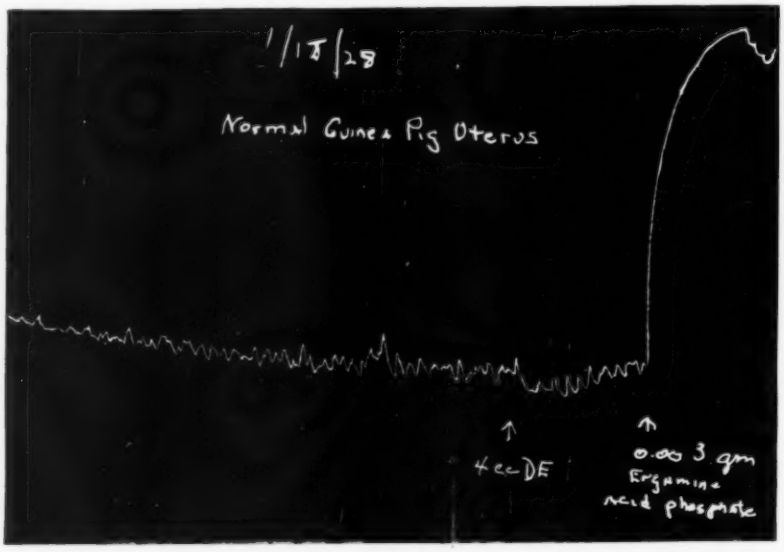

Fig. 1.

was crystal clear and sterile before use for intravenous injection. We therefore believe that our solutions measure up to the criteria laid down by Hanzlik and Karsner.[5]

We previously found that dander extract (DE) was toxic in amounts over 1.5 c.c. when used intravenously and we therefore never used it in amounts over 1 c.c.[4] When used in the Dale experiments amounts up to 4 c.c. are not toxic, as is demonstrated in Fig. 1. In our experiments we have usually used 1 c.c. and never more than 2 c.c.

Methods.—

The methods are described in detail as they were carried out.

Intravenous Injection.—The animal's head is held in place by an Auer clamp, and the legs grasped firmly. There is less tension if the legs of the animal are held by an assistant than if the animal is fastened to an animal board. Though the neck is taut and the animal is unable to struggle, it can

breathe comfortably. An incision is made in the midline of the neck and the jugular vein exposed. The solution is then injected slowly from a fine hypodermic needle into the jugular vein toward the heart. As the needle is removed the vein is grasped with a hemostat, lightly held, and tied off with a linen thread. The neck wound is then sutured.

2. *Intraperitoneal Injection.*—The solution is injected directly into the intraperitoneal cavity, halfway down the abdomen to one side of the midline. Care must be taken not to puncture the urinary bladder which may be distended. It is practically impossible to puncture the gut.

3. *Inhalation (Respiratory Anaphylaxis).*[4]—The animal is placed on a false wire floor in a glass cage (see Fig. 2). A fine suspension of dust is thrown up

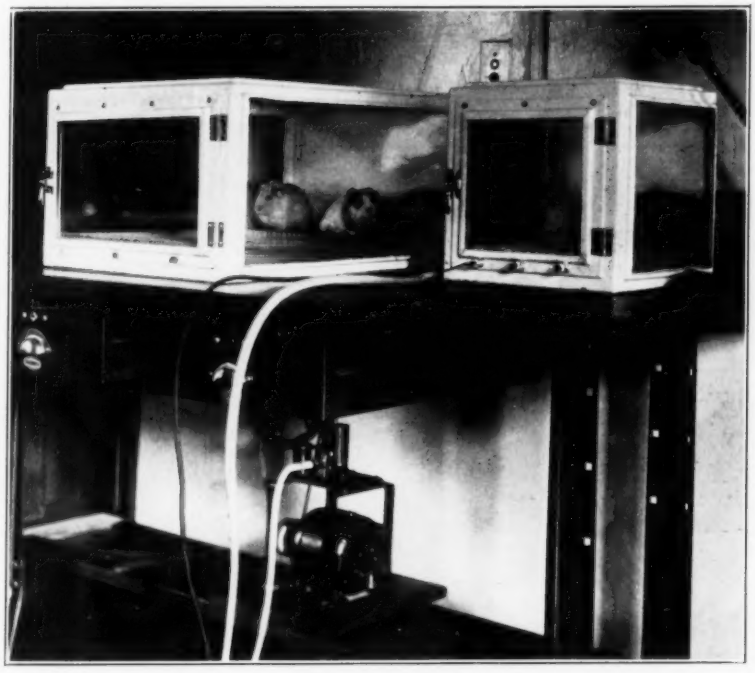

Fig. 2.

from the true floor of the cage by alternating puffs of air blown in by a motor-driven pump. The dust circulates in only moderate density, causing the animal to inhale it in much the same manner as do human beings in contact with dust. It is essential that the dust be absolutely dry and devoid of fat in order to insure circulation of the dust and free passage into the nares of the animal.

4. *Uterine Strip—Schultz-Dale.*—The animal is struck a blow on the back of the head, placed on an animal board and the abdomen opened wide. A loop of thread is passed through the upper pole of one uterine horn and tied with a double surgeon's knot. Another thread, attached at one end to a glass tube, is run through the uterus about 1 cm. below the first attachment and is also looped and firmly fastened. The entire procedure of attaching the

uterine strip to the threads was carried out with the uterus in situ; the uterus is thus bathed in the body fluids, instead of being placed in a bath of warm Locke's solution. This method is more rapid, results in less trauma and less exposure of the muscle. The uterine horn is then severed above and below these attachments, carefully removed from the abdomen and immediately immersed in a 250 c.c. bath of oxygenated Locke's solution at 37° to 40° C. The glass tube is set into a clamp and the loose thread which is tied to the upper pole, is attached to a recording lever. Throughout the procedure, from the severing of the uterus to the setting up in the bath, as little tension as possible is put on the threads. The writing point of the lever is set on a revolving smoked drum; a minute-marker records the time.

When a base line is reached, the uterus having assumed its normal rhythmic contractions, antigen is placed in the bath and the contractions are recorded on the smoked drum. Desensitization may be studied by placing antigen into a changed bath after the contraction is completed and a normal

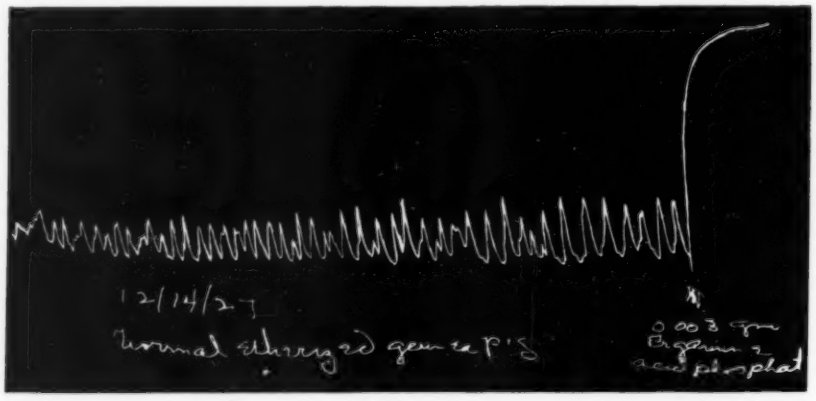

Fig. 3.

base line again resumed. After these procedures are completed, 0.001 gm. ergamine acid phosphate is dropped into the bath to elicit a maximal normal contraction. This step is essential to ascertain whether the muscle is still able to contract, for without this information, a negative response cannot be evaluated.

5. *Double-Shock Method.*—For comparative purposes we use what we call the "double-shock method." That is, the original shock dose is followed in an hour by an intravenous injection.

The methods used were:

(a) the intraperitoneal—intravenous

(b) the inhalation—intravenous

(c) the uterine strip—intravenous

(a) and (b) require no further description.

6. *Uterine Strip—Intravenous Method.*—For (c) we used the following modification: Instead of killing the animal, the previously sensitized guinea

pig is placed on an animal board and etherized. A laparotomy is performed, the uterus removed in the manner described above, and the complete uterine strip procedure carried out. The laparotomy wound is then sewed up and the animal allowed to recover fully from anesthesia. One hour later the intravenous injection is given in the usual manner. To show that ether anesthesia has no effect on the uterine strip, the tracing made by the uterine horn of an animal at the height of anesthesia (Fig. 3) can be compared with the response of the uterine horn of a normal animal to ergamine acid phosphate (normal uterine contractor) which is shown in Fig. 4. Since the uterine horn in Fig. 3 shows a normal response to ergamine acid phosphate it may be assumed that anesthesia does not affect the contractility of the uterine strip. Further, in our experiments we have had numerous examples of animals exhibiting anaphylactic shock following the intravenous injection after recovering from ether anesthesia.

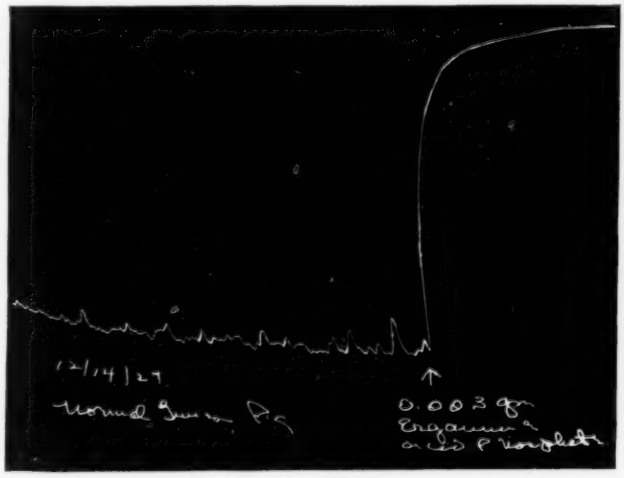

Fig. 4.

Criteria of Anaphylactic Shock.—

Dyspnea of varying degrees, marked scratching, convulsions, collapse, and in certain instances ocular manifestations[6] are accepted as symptoms of anaphylactic shock. The best criterion, however, is acute anaphylactic death occurring within a few minutes after injection. If in a given set of experiments such deaths do occur the other symptoms can be accepted as evidence of varying degrees of anaphylactic shock. The lungs of an animal dying in acute anaphylactic shock are completely emphysematous, because of spasm of the smooth muscle of the bronchioles. Such lungs float in ether or water. Small hemorrhagic areas may or may not be present. Any deviation from the above pathologic picture makes the diagnosis questionable.

Animals that die after a protracted period must be placed in the doubtful category. There were but few instances of this kind in many hundreds of

experiments. One example was that of an animal which at necropsy showed pulmonary pathology, pericarditis and adhesive pleurisy.*

Comparison Between the Intraperitoneal and Intravenous Shock Doses.—

TABLE I

COMPARISON BETWEEN THE INTRAPERITONEAL AND INTRAVENOUS ROUTES IN THE SAME ANIMAL

NUMBER OF ANIMALS	ANAPHYLACTIC REACTION AFTER INTRAPERITONEAL INJECTION	ANAPHYLACTIC REACTION AFTER INTRAVENOUS INJECTION
4	−	+ + + +
2	+ −	+ + + +
3	+	+ + + +
5	+ + +	+ + + +
3	−	+ + +
1	+	+ + +
5	−	+ +
1	+ −	+
1	+	+
21	−	−

− No anaphylactic reaction
+ − Doubtful reaction
+ Showing dyspnea and scratching
+ + Showing marked dyspnea, convulsive movements
+ + + Showing severe dyspnea, convulsions, collapse, final recovery.
+ + + + Typical anaphylactic death with completely ballooned lungs, occurring within a few minutes after injection

In Table I are the results of the intraperitoneal-intravenous comparison. After a suitable incubation period, 46 animals weighing from 50 to 820 gm. were given intraperitoneal injections. One hour later the surviving animals were injected intravenously.

From the results obtained it would appear that while the intraperitoneal route may serve as a diagnostic procedure in the determination of an anaphylactic state it is not always accurate. Animals which were entirely negative or showed anaphylactic symptoms and recovered after intraperitoneal injection, did die in shock after intravenous injection. Although there may be a feeling that previous injection renders the animal more sensitive to the intravenous injection, the fact that at least 20 animals gave negative responses to both the intraperitoneal and intravenous injections would indicate this objection to be invalid.

Comparison Between the Inhalation and Intravenous Shock Doses.—

In a previous study[4] we showed that sensitized animals die in anaphylactic shock from the inhalation of dusts in as short a time as with intravenous injection. Therefore, as in the above group, we gave intravenous injections only to those animals which reacted negatively to the inhalation of dusts or recovered from respiratory anaphylaxis induced in this manner. From Table II it is quite evident that the intravenous method is more accurate in evaluating the state of hypersensitiveness than is the inhalation method.

*A caution that might be advised at this point is the source of animal supply; for one must be certain of the integrity of the animal dealer so that there is no question of the normalcy of the animal.

TABLE II

COMPARISON BETWEEN THE RESPIRATORY AND INTRAVENOUS ROUTES IN THE SAME ANIMAL

NUMBER OF ANIMALS	ANAPHYLACTIC REACTION AFTER INHALATION	ANAPHYLACTIC REACTION AFTER INTRAVENOUS INJECTION
3	−	++++
1	+−	++++
4	+	++++
9	+++	++++
2	+−	+++
1	+	+++
3	+	+
2	+−	+−
4	−	−

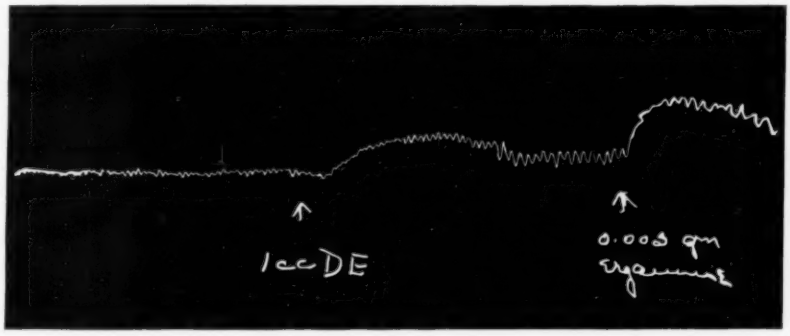

Fig. 5.—Animal No. 1061 (420 gm.) sensitized on May 9, 1928, by an intraperitoneal injection of dander extract (DE). On June 28, 1928, the animal was etherized and uterus taken out for Dale. This is an example of a very inactive muscle; however, it did give a weak but definite response on addition of 1 c.c. DE to bath and a similar response to the normal uterine contractor ergamine acid phosphate. When the animal came out of the ether, it was given an intravenous injection of 0.5 c.c. DE and promptly died in typical anaphylactic shock. Lungs were typical.

Comparison Between the Uterine Strip and Intravenous Methods.—

Before examining the results of this comparison, it may be interesting to analyze a few of the tracings which are presented.

In Fig. 5 we have an example of a weak uterine strip, although it was taken from a medium-sized animal and after a fairly long incubation period. The response could be evaluated accurately only in view of the positive reaction of the animal after intravenous injection.

An excellent type of uterine strip is illustrated in Fig. 6.

Fig. 7 shows an extremely active muscle. It was taken from an animal weighing only 340 gm. which is not generally considered too large. The positive contraction in this tracing can only be interpreted when compared with the contraction obtained by the use of ergamine. With such muscles all degrees of hyperactivity are found and are most difficult to interpret. The evaluation is based on the plateau arrangement of the tracing and a comparison with the subsequent normal ergamine response.

An example of a uterus which responds erratically and peculiarly is illustrated in Fig. 8. It is almost impossible to interpret results in such instances.

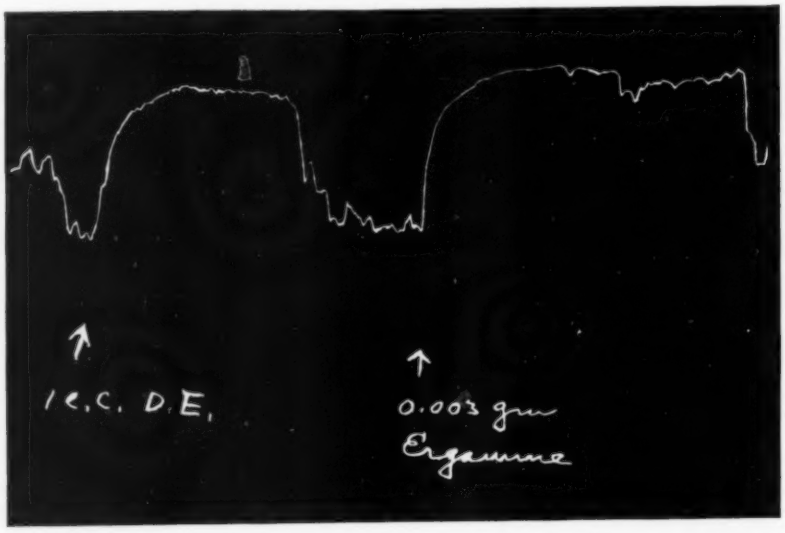

Fig. 6.—Animal No. 1052 (295 gm.) sensitized on Mar. 21, 1928, by an ip. injection of 2.0 c.c. DE. On Apr. 10, 1928, the animal was etherized and the uterine strip removed. In the above tracing the first arrow indicates the addition of 1 c.c. DE to the bath; this caused a definite contraction of the muscle. After a regular base line had been reached, 0.003 gm. ergamine acid phosphate was added to the bath and a contraction elicited which was of practically the same magnitude as the former. After the animal had come out of the ether, about one hour later, it was given an intravenous injection of 0.1 c.c. DE and promptly died in typical anaphylactic shock. The lungs were typical.

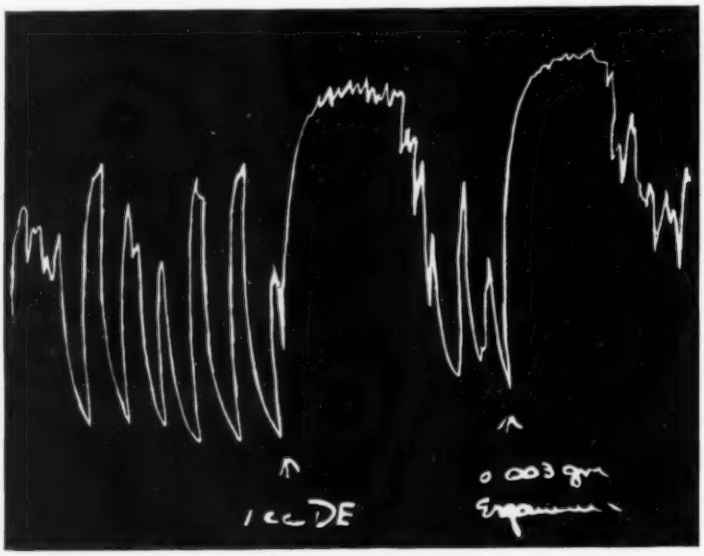

Fig. 7.—Animal No. 1063 (340 gm.) sensitized by an ip. injection of 2.0 c.c. DE on May 9, 1928. On June 28, 1928, the animal was etherized and the uterine strip taken out for Dale. This is an example of a hyperactive muscle. However, on addition of 1 c.c. DE to the bath, it gave a specific response with plateau contraction; on addition of ergamine, a normal uterine contractor, it gave a similar plateau contraction. When the animal came out of the ether it was given an intravenous injection of 0.5 c.c. DE and promptly died in typical anaphylactic shock. Lungs were typical.

An excellent uterine muscle reaction is illustrated in Fig. 9. The negative reaction is confirmed by a negative response of the animal to intravenous injection.

Analysis of results summarized in Table III indicates that the Schultz-Dale uterine strip reaction as a criterion for hypersensitivity is less reliable than the intravenous method. These irregularities may be accounted for by the variability of the nature of the individual uterine muscles.

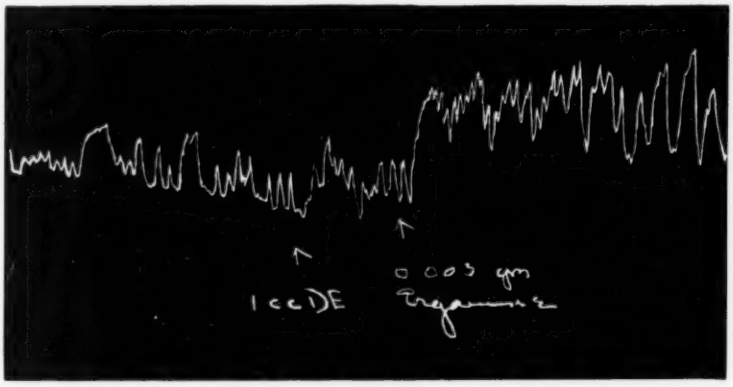

Fig. 8.—Animal No. 1070 (365 gm.) sensitized by an ip. injection of 2.0 c.c. DE on May 31, 1928. On July 24, 1928, the animal was etherized and uterine strip taken out for Dale. On addition of 1 c.c. DE to the Dale bath no definite reaction was obtained nor did the muscle react definitely to the addition of 0.003 gm. ergamine acid phosphate. However, on intravenous injection of 0.5 c.c. DE the animal died in typical anaphylactic shock.

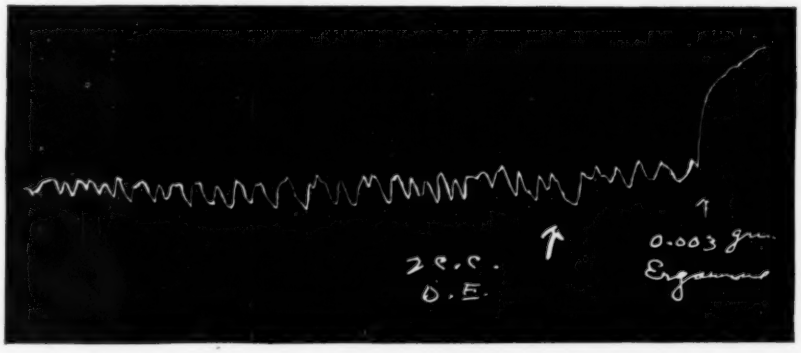

Fig. 9.—Animal No. 1276 (270 gm.) sensitized on Jan. 4, 1928, with 4 c.c. DE ip. On Jan. 24, 1928, the animal was etherized and uterus taken out for Dale experiment. After the animal came out of the ether, it was given an injection of 0.5 c.c. DE intravenously and was negative. In view of this, the reaction of the uterus on addition of 2.0 c.c. DE to bath is rather interesting. It showed only a negligible depression followed by no elevation and, since the addition of ergamine caused a contraction, the inference is that the animal was not sensitive.

TABLE III

COMPARISON BETWEEN THE UTERINE HORN REACTION AND INTRAVENOUS INJECTION IN THE SAME ANIMAL

NUMBER OF ANIMALS	UTERINE HORN REACTION (DALE)	ANAPHYLACTIC REACTION AFTER INTRAVENOUS INJECTION
11	Positive	Death
1	Doubtful	Death
6	Negative	Death
5	Negative	Negative

COMMENT

That a state of hypersensitiveness can be demonstrated by either the intraperitoneal, inhalation or uterine methods is unquestioned, but we believe that the experiments detailed above justify the assumption that the intravenous method for inducing anaphylactic shock when properly done is a method of greater accuracy. The comparison of these methods was accomplished by the utilization of what may be termed "double-shock method." In order to make the comparison between the intravenous and uterine strip method we modified the original Dale method. Instead of killing the animal it was anesthetized, the uterine strip removed, and the regular Dale method performed. When the animal completely recovered from anesthesia an intravenous injection was given. Our comparisons show that the intravenous route gives more consistently accurate results than the Schultz-Dale.

A similar modification was described by Walzer and Grove[7] except that no anesthesia was used. In their method anesthesia is interdicted because the intravenous injection is given immediately after the uterine strip is removed, but anesthesia does not interfere with the uterine strip reaction or the subsequent demonstration of shock, in our modification of their method.

Under favorable conditions the uterine strip cannot be used because of the irritability and viability of the muscle. Therefore animals in heat, or with weak and traumatized muscles cannot be used. The availability of proper animals is therefore narrowed down to a certain ideal type. In experiments where it is necessary to use various sized animals, ranging from the newly born to the adult or pregnant pig, the usefulness of the Dale ceases. Another factor of great importance is the time consumed in performing a Dale experiment. For general use, therefore, and particularly where numerous experiments are being done, the intravenous method is superior to the Dale because of its greater expediency and regularity.

It is proposed that when the uterine strip method is to be used for graphic demonstration or for eliciting sensitization to more than one substance the modification of the original Dale be used, thereby utilizing the uterine and intravenous methods in the same animal with its obvious advantages.

The intravenous is the method of choice in experiments on dogs, rabbits and monkeys and we believe should be accepted as the best method in guinea pigs as well.

Our work does not in any way militate against the acceptance of a positive reaction with the subcutaneous intraperitoneal and inhalation or Dale method. It does, however, challenge the validity of a negative reaction when the above 4 methods are used, and of a positive reaction when the intracardiac or intrathecal methods are used. Many investigators using one or more of these 6 methods obtained results which might have been more conclusive had they used instead the carefully controlled intravenous method.

SUMMARY AND CONCLUSIONS

1. Comparison of the intraperitoneal, respiratory, and uterine strip respectively with the intravenous route for the demonstration of anaphylaxis has been made by the utilization of the "double-shock method."

2. A modification of the Dale method for uterine strip reaction is described.

3. Negative or suggestive anaphylactic reactions obtained by the intraperitoneal respiratory or uterine strip methods are shown in certain instances to be positive by the intravenous method.

4. We therefore believe that the intravenous shock method properly performed is superior to all other methods for accurately determining a state of hypersensitiveness in the guinea pig, as well as in other animal species.

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ELECTROCARDIOGRAPHIC STUDIES ON CHICK EMBRYO HEARTS*

I. A Technic for Recording Electrical Changes in Isolated Chick Embryo Hearts

By Herbert Pollack, M.D., Boston, Mass.

THE isolated intact warm-blooded heart under suitable conditions lends itself to many experimental procedures. The literature contains a few references to the normal electrocardiograms of developing chick embryos (Cluzet et Sarvonat, 1914); (Wertheim-Salmonson, 1913); (Spadolini and de Georgis, 1921); (Sands, 1929). There is, however, a surprising lack of reports on experimental alterations of the normal. It was thought that a technic of recording the electrical changes in the isolated warm-blooded (Pollack, 1930) heart would offer possibilities of determining the direct effects of many drugs. This should alleviate much of the criticism directed toward the work on cold-blooded hearts as not pertaining to the warm-blooded. The plan of this work is to study (a) the functional changes as recorded by the electromyograms; (b) chemical changes by subsequent analyses; and (c) histopathologic changes.

Toward this end the following technic was devised and has proved to work satisfactorily. Fertile chicken eggs are incubated to the desired stage. The embryos are removed from the shell and pinned out on a paraffin block. The heart is dissected out carefully, leaving a fairly long stalk of great vessels and some tissue. This avoids injury to the auricles and allows for handling. With practice the time taken for this procedure is negligible. The shock during this operation may sometimes cause the heart to stop beating temporarily. The normal rhythm is usually reestablished when the heart is placed in a warm incubator. The receptacle for the heart is made of an oblong glass dish about 2 cm. deep. This is filled with paraffin. A hole is excavated in the paraffin approximately $2 \times 1 \times 1.5$ cm. The dish is cemented to an ordinary microscopic slide which is set on a mechanical stage.

The electrodes for leading the current from the heart must be chosen for (1) nonpolarizable character, (2) low resistance, and (3) small surface for contacts. The type finally chosen as being most suitable for this work is a modified zinc-zinc sulphate cell. An ordinary culture tube is drawn to a capillary about three inches from the mouth. The constricted portion between the capillary tip and body of the tube is bent out at about a 60° angle. The capillary tip is then bent down in the complementary angle at a point where it extends beyond the outer wall of the tube, and cut to leave a tip of a few millimeters. This is done to allow approximation of the tips of the two

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electrodes. A glass slide is cemented to the body of the tube with DeKhotensky for fixation to mechanical stages. The capillary tip is threaded with several thicknesses of cotton string. These should be snugly packed in the capillary opening. Too tight a fit interferes with capillarity, causing poor wetting, which increases the resistance. The thread should be run well up into the body of the tube. The outside string is cut about 0.5 cm. from the opening and wound with a spiral thread. Sodium chloride solution (0.9 per cent) is used to saturate the string, which serves as the immediate contact with the heart. The tube is filled with a thin paste of kaolin and saturated zinc sulphate. The zinc poles are pushed as far down into the tube as possible. By

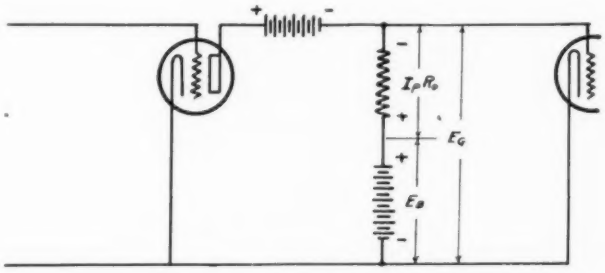

Fig. 1.

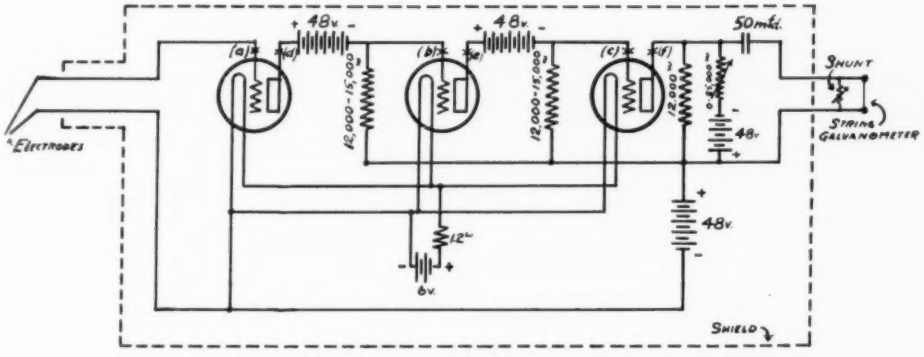

Fig. 2.

grinding and bending the distal ends they may be fitted into the curves. The tubes are stoppered by corks bored for the poles and sealed with thick celloidin. The electrodes have a resistance varying from 2000 to 4000 ohms. They can be used for constant recording over periods of several hours without showing an appreciable amount of polarization or resistance changes. Owing to the action of the zinc sulphate on the cotton, it is advisable to renew the filling every eight weeks.

In order to maintain the hearts at proper temperature, the electrodes were set up inside an incubator. An iron bar three inches wide, three-quarters of an inch thick, and long enough to reach across the incubator is tapped out for three vertical one-half inch steel rods. The two taller rods are set about two inches from each end, and a shorter one set halfway from the cen-

ter to one end. Another rod is clamped rigidly across the two end vertical ones at the desired height. The base bar is rigidly clamped to the incubator shelf. The mechanical stages with the electrodes are clamped to the cross-bar. The mechanical stage with the chamber is clamped to the middle short vertical bar by means of a short side arm. This gives a three dimensional range of motion in placing the electrode in relation to the heart.

The use of an electrolyte medium for bathing the heart introduces a very important factor in the electrical circuit. Any saline medium flooding the chamber acts as a shunt across the electrode tips and tremendously reduces the recorded voltages. The fact that the cotton tips of the electrodes have the same resistance as the medium in which they are bathed prevents any efficient alterations at that place. There are two ways around this difficulty: (1) to use a "dry" heart, or (2) develop a device to amplify the recorded potentials.

For the work on the developmental aspect of the electrocardiogram of the embryo the dry heart was used. For the experimental work in determining the effect of drugs, where a saline medium is necessary, an amplifier was developed. A description of this will soon be published.

Alternating currents are one of the most disturbing factors encountered in the development of the technic. These can be reduced to a minimum by complete shielding. If an electric incubator is used, all wires must be entirely disconnected by removing the plugs from the sockets during the time when the string galvanometer is in the circuit. If the lighting current is of the A.C. type, it is preferable not to have any bulbs lit in the incubator room.

The cable to the string galvanometer is run directly into the incubator and the wires connected to the zinc poles. The connections are waterproofed with several coats of Ambroid cement. The danger of exposed unions of two dissimilar metals lies in the condensation of water vapor and the setting up of currents between the copper and zinc. This results in a wandering string. The metal coating of the cable must be grounded at one end at least.

When the bare heart is used proper moisture conditions are maintained by saturating the air of the incubator with water vapor. A shallow enamel pan of the type used in photography is placed on the bottom of the incubator. This is filled with water. An immersion "Hot Point" heater is set in the water and the cord run out through a ventilating hole in the wall of the incubator.

The cooling of the incubator when the heating currents are off is minimized if the pan of water has been brought to a boil before disconnecting the current.

Three leads from the heart are feasible in embryos over four days. The attempt was made to keep them referable to the usual clinical leads. Lead α taken across the two auricles, lead β is taken with one electrode on the right auricle and the other at the apex, γ is left auricle and apex. The records of various stages are consistent when taken at different times. Minor variations in the position of the apical electrodes in leads β and γ do not affect the fundamental picture.

SUMMARY

A technic is described for obtaining electrocardiographic tracings from the isolated chick hearts. The various difficulties which may be encountered are outlined with instructions for avoiding them.

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A CASE OF OCCLUSION OF BOTH CORONARY ARTERIES WITH RUPTURE OF THE AURICLE*†

By James R. Lisa, M.D., and Alfred Ring, M.D., New York, N. Y.

BILATERAL occlusion of the coronary arteries is an excessively rare condition. The most frequent site of the lesion is at the coronary mouths usually caused by syphilitic aortitis, as in the two cases recently reported in the contribution of Leary and Wearn[1] in which they review the literature. Occlusion beyond the mouths is an even more unusual condition, only two cases being quoted in the article mentioned. In the case to be reported the occlusion occurred beyond the mouths and one auricle was the site of a recent tear through its muscular wall, a finding we believe to be unique.

CASE REPORT

The patient, fifty-three years old, Hebrew, was first admitted to City Hospital March 15, 1928. He had always been well until four years previously. Until six years before he had been a heavy whisky drinker; the family and personal histories otherwise were irrelevant. The onset was with epigastric pain relieved by vomiting or the administration of alkalies. During the ensuing four years the symptoms persisted; an appendectomy had been performed with temporary relief. About a year after the onset he suffered a temporary hemiplegia. During the last year the abdominal symptoms had subsided somewhat, but substernal pain appeared induced by slight exertion, radiating to the left arm and shoulder, associated with moderate dyspnea, and relieved by rest.

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†Presented before the Medical Society of the New York Polyclinic Medical School and Hospital, Dec. 1, 1930.

On admission to the hospital the heart was slightly enlarged to the left, the sounds were distant and valvular in quality, rate 82 with no deficit or irregularity, and the blood pressure was 136/90. The right upper quadrant was tender; the liver was not palpable. The epigastrium was tender and slightly rigid. In the lumbar region was a point of tenderness. The urine, serology, and blood chemistry were normal. The roentgenographic diagnoses were moderate cardiac enlargement and duodenal ulcer.

During the next three months he improved greatly on a Sippy diet. On two occasions, however, he had attacks of severe epigastric pain, sudden in onset, remaining localized, and lasting about one hour. Examination of the heart revealed no changes from previous findings. EKG. (Dr. H. E. B. Pardee) May 22, 1928, a day previous to one attack: Normal sinus rhythm, normal A-V

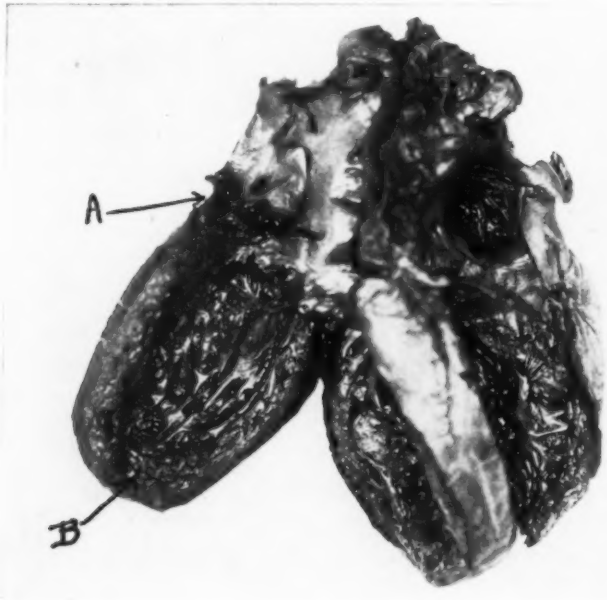

Fig. 1.—The gross appearance of the heart. Arrow *A* indicates the site of the rupture of the auricular wall. Arrow *B* indicates the old infarcation at the apex and the mural thrombus.

conduction time, neither right nor left axis deviation, QRS wide and notched in all leads, abnormal inversion of T-wave in Leads I and II, a suggestion of coronary T-wave in Lead III.

For one month he remained well. Then the gastric symptoms again recurred, were definitely associated with meals, coming on in two hours, and were relieved by the same methods he had previously employed. Gastroenterostomy was performed and, after a stormy convalescence, he was discharged in October.

From then until February, 1929, when he was readmitted, there had been but slight amelioration of his symptoms. The physical signs remained unchanged. The roentgenograms and fluoroscopy revealed good function of the stoma, no tenderness, spasticity of the pylorus, irregularity, enlargement and spasticity of the duodenal cap. He remained under observation only a short time and was discharged unimproved.

In April, 1930, he again entered the hospital. During the interval the symptoms had persisted, become more severe, and dyspnea was more prominent. On the night of admission he had an attack of severe epigastric pain lasting about one hour, with marked dyspnea; orthopnea; cyanosis of the lips, fingers, and face; cough, and blood-tinged sputum. The heart was distinctly larger than previously, and irregular in rate and force; there was a pulse deficit and the blood pressure was 128/86. EKG. (Dr. G. J. Chandlee) April 27, 1930; normal sinus rhythm, normal A-V conduction time, neither right nor left axis deviation, notched QRS in all leads, an unusual peculiarity of the QRS group in that Q is notched and overlarge, and an abnormal inversion of T-wave in Leads II and III.

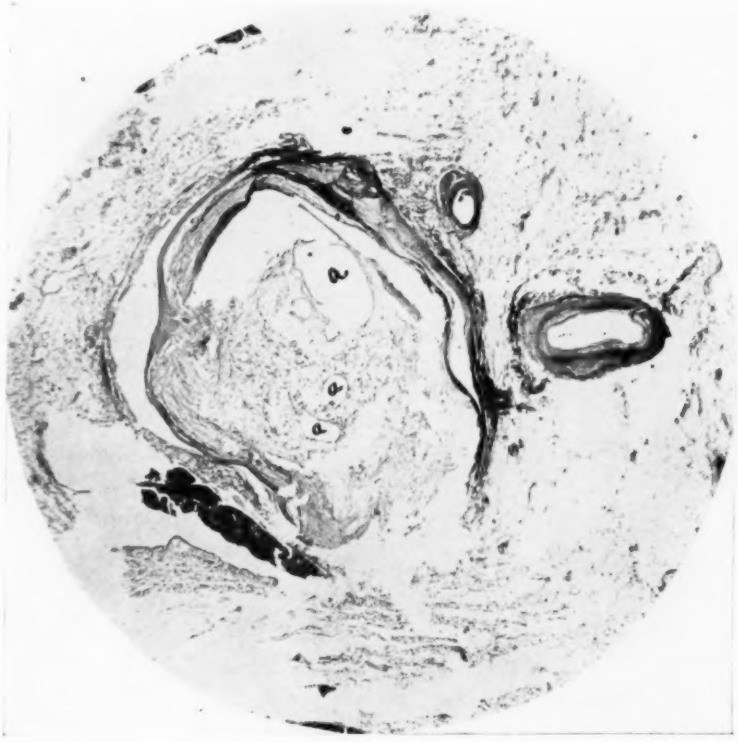

Fig. 2.—Cross-section of the right coronary artery. Letters "a" indicate canalization. The small arteries reveal the changes found in many small branches. The left coronary artery was similar.

From then until his death in June the course was rapidly progressive. Attacks of pain were frequent and severe and soon became localized to the precordium. Sometimes they were associated with signs of shock, such as grey cyanosis and perspiration. The heart decompensated, cyanosis increased, pleural effusion appeared, and a gallop rhythm developed. Toward the end he presented a dramatic picture of intense air hunger and almost constant precordial pain.

Heart: Gross Appearance.—The heart was dilated and hypertrophied, weighing 775 grams. The mouths of both coronary arteries were slightly nar-

rowed and quite markedly sclerotic. The left coronary beyond the mouth was moderately arteriosclerotic and the lumen was slightly narrowed. From the origin of the anterior descending branch throughout its course in the auriculoventricular groove, it was intensely sclerotic and apparently completely occluded by well-organized thrombus. In the upper portion of the anterior descending branch was a fresh thrombus. The other branches coursing over the left ventricle were markedly sclerotic and the lumina narrow but patent. The right coronary artery was very similar to the left. Its branches were somewhat less sclerotic and had no thrombi. All the chambers had thick fibrosed walls. In the right ventricle the scarring was widespread but there were no large areas of fibrosis. The left ventricle and interventricular wall were tremendously scarred; near the apex was a massive fibrosis covered on its inner

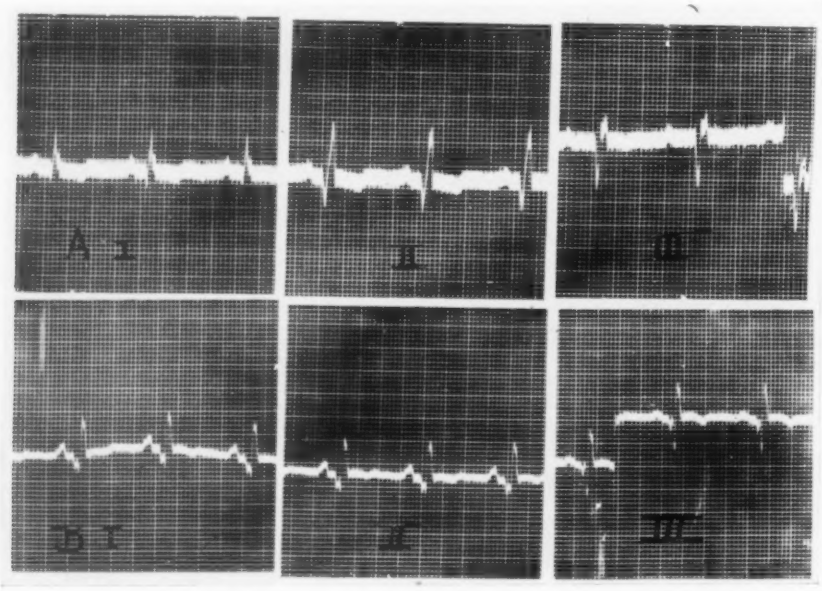

Fig. 3.—EKG tracings. (*A*) taken May 22, 1928. (*B*) April 27, 1930.

surface by organized mural thrombus. In the left auricle just above the base of the posterior mitral cusp was a fresh rent 8 cm. long through the wall to the epicardial fat and it was filled with fresh clot. The left coronary artery lay in the base and the small branches were pipe stem and occluded.

Microscopy.—Sections through both arteries showed intense arteriosclerosis and complete organization of thrombus with marked canalization, greater in degree in the left than in the right. The more superficial branches were markedly sclerotic but the lumina were relatively large. The fibrosis in the auricles and right ventricle was diffuse and definitely associated with the nutrient arteries. The more fibrosed arteries lay in the heavier scars. In the interventricular wall and left ventricle the scarring was most marked in the central portion of the muscle; the subendothelial layer was remarkably preserved and showed acute degenerative changes. The fibrosis was of all ages. In the

region of the rent the scarring was intense, the nutrient vessels being sclerotic with greatly narrowed lumina; the muscle fibers were foamy and granular and the hemorrhage extended well beyond the margins. The conduction system was curiously well preserved. In the region of the sinoauricular node was slight scarring, moderate arteriosclerosis, and a mild mixed cell infiltration of polynuclears—plasma lymphocytes and endothelial leucocytes—beneath the epicardium. The nodal tissue was normal. The atrioventricular node was penetrated slightly at its periphery by scar tissue. The bundle was normal. The right branch was uninvolved, the deeper scars reaching it in a few areas only. The left branch was acutely degenerated as was the adjacent musculature in this region.

The pyloric portion of the stomach and the duodenum had no evidence of ulcer and showed only a mild chronic lesion both on gross and microscopic examination.

SUMMARY

A case is reported of arteriosclerotic occlusion of both coronary arteries with rupture of the auricle. The clinical course was that of coronary disease with the anginal symptoms referred to the upper abdomen and only comparatively late becoming precordial in localization. The lesions indicate an extremely slow progress of the disease.

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REMOVAL OF SERUM PROTEIN BY MEANS OF ELECTRICITY*

By A. Tasman, Ph.D., and A. C. Brandwijk, M.D., Utrecht, Holland

FROM the hand of G. Turner[1] there appeared some time ago a publication regarding the removal of protein material from the blood serum by means of electricity. As a cathode he used a small piece of platinum sheet and as an anode a small sheet of gold. No further particulars of the method were to be found in his report than the information of the electrical intensity, ranging from 30 to 8 milliampère and an electrical tension of 100 to 20,000 volts.

According to this method, antibodies (amboceptor), complement, etc., should be obtained totally free from protein, be precipitated out of the liquid by means of a weak acid and after centrifugation, redissolved in slightly alkaline physiologic salt solution.

Considering the great practical as well as theoretical importance which this manner of operating could have as regards purification, respectively concentration of antitoxic sera, the method was applied to an antidiphtheritic serum.

*From the Rijks-Serologisch Instituut, Utrecht, Holland, Dir. Prof. Dr. H. Aldershoff.
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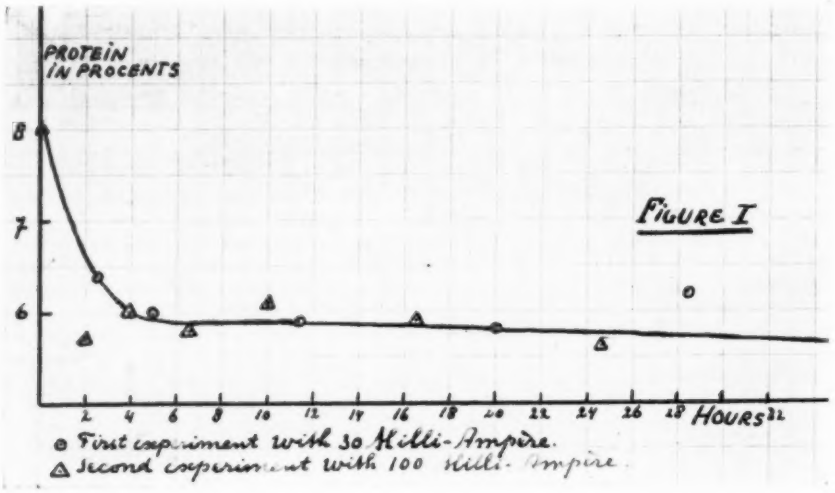
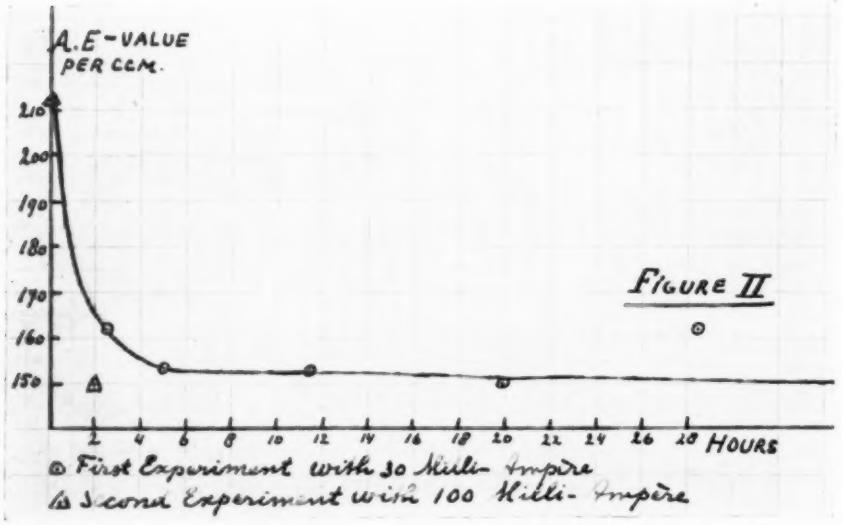
Before passing on to the experiments a small apparatus was made as indicated by Turner. After an electrolysis of about twenty-four hours we observed a very marked decrease of the antitoxin content, but the liquid was by no means free from protein.

To be able to employ larger quantities, electrodes were made of fine platina-netting with a surface of 4 × 15 cm.[2] These electrodes were placed in


PROTEIN IN PROCENTS
8
7
6
2 4 6 8 10 12 14 16 18 20 22 24 26 28 HOURS 32
FIGURE I
○ First experiment with 30 Milli-Ampère.
△ Second experiment with 100 Milli-Ampère.



a glass beaker of 1 liter with a distance between them of 8 cm. During the electrolysis, the serum was kept in motion by an electrically driven stirring apparatus. At fixed times a quantity of about 100 c.cm. was taken out and filtered through a "Seitz Entkeimungsfilter," which had been sterilized in the autoclave. We determined the P_H, the strength (expressed in A.U., antitoxic units) per c.cm. according to the flocculation reaction of Ramon[2]

and also the percentage of protein. This last value was obtained by means of two Kjeldahl-determinations, the total percentage of nitrogen and the non-protein nitrogen, found in the filtrate of the serum made free from protein with the aid of trichloracetic acid.[3] From the difference between the last two values, the protein nitrogen was found, which multiplied with 6.25 gave the percentage of protein.

Two experiments were performed, one with an electric current of 30 milliampère and another with 100 milliampère. The statement of Turner reporting a tension of 100 to 10,000 volts must be an error. A tension of 10,000 volts is not to be obtained considering the comparatively small electrical resistance of the serum and the small distance between the electrodes.

The results are given in Tables I and II. Fig. 1 shows the course of the diminution of the protein material during the test and Fig. 2 indicates the decrease of antitoxin. Although the percentages of protein show more fluctuation in the second test than in the first one (probably caused by E.K.-filters more or less humid), yet they seem to fit the same curve.

TABLE I

CURRENT INTENSITY 30 MILLIAMPÈRE

TIME OF THE EXPERIMENT	TEMPERATURE	P_H	TOTAL NITROGEN*	NONPROTEIN NITROGEN*	PROTEIN CONTENT	STRENGTH ACCORDING TO RAMON
					PER CENT	
0 hours	17.9°	7.5	1.305	0.032	8.0	202 A.U. per c.cm.
2½ hours	18.8°	7.7	1.048	0.025	6.4	161 A.U. per c.cm.
5 hours	19.0°	7.7	0.980	0.023	6.0	153 A.U. per c.cm.
11½ hours	19.0°	7.8	0.962	0.022	5.9	153 A.U. per c.cm.
20 hours	19.0°	7.8	0.942	0.022	5.8	150 A.U. per c.cm.
28½ hours	19.0°	7.9	1.011	0.015	6.2	161 A.U. per c.cm.
44 hours	19.0°	7.9	0.970	0.014	6.0	Does not flocculate

TABLE II

CURRENT INTENSITY 100 MILLIAMPÈRE

TIME OF THE EXPERIMENT	TEMPERATURE	P_H	TOTAL NITROGEN*	NONPROTEIN NITROGEN*	PROTEIN CONTENT	STRENGTH ACCORDING TO RAMON
					PER CENT	
0 hours	14.8°	7.5	1.305	0.032	8.0	202 A.U. per c.cm.
2 hours	15.3°	7.6	0.911(?)	0.024	5.7	150 A.U. per c.cm.
4 hours	16.5°	7.7	0.985	0.024	6.0	Does not flocculate
6½ hours	—	7.7	0.956	0.024	5.8	Does not flocculate
10 hours	14.6°	8.0	0.998	0.024	6.1	Does not flocculate
16¼ hours	15.5°	8.3	0.962	0.024	5.9	Does not flocculate
24¼ hours	18.4°	8.4	0.914	0.024	5.6	Does not flocculate

*Expressed in grams nitrogen per 100 c.cm. of serum.

The results obtained indicate that but 25 or 30 per cent of the protein material is separated from the serum. There is a parallel drop of the antitoxin content. The purification of antitoxic sera by the method indicated by Turner, performed in the manner prescribed by him, is not possible.

Either Turner has made an error in determining the percentage of protein and antibodies of his serum or the method followed by him differs considerably from the one described above.

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LABORATORY METHODS

A SIMPLE METHOD FOR ESTIMATING THE TOTAL PRECIPITABLE PROTEIN IN CEREBROSPINAL FLUID*

By A. Robert Bauer, M.D., and H. P. Schenck, M.D., Philadelphia, Pa.

A SIMPLE method for estimating the total precipitable protein in cerebrospinal fluid is desirable, since most pathologic conditions of the central nervous system are accompanied by an increase of the protein content of the cerebrospinal fluid, and the detection of such increases may aid or corroborate diagnosis. The technic, described below, has sufficient accuracy for general clinical purposes and does not require a skilled or experienced laboratory technician for the interpretation of results.

It has been customary for most laboratories to employ methods for quantitative estimation of total protein in the spinal fluid as a routine procedure, but the methods have proved only roughly quantitative and depend for accuracy of reading upon the experience of the technician. The methods of Nonne-Apelt,[1] Noguchi,[2] Pandy,[3] and Weichbrodt[4] are merely globulin estimations and give no information as to the amount of albumin present. Levinson,[5] Greenfield,[6] Mestrezat[7] and others disagree as to whether the increase in the protein content of pathologic fluids is in the albumin or globulin fractions. Obviously, if it be in the albumin fraction, as claimed by Mestrezat, the globulin tests are wholly inadequate.

Many methods have been devised for estimating total protein. Ravaut's "Albuminodosimètre,"[8] the method of Denis and Ayer[9] and Mestrezat's diaphanometric method depend upon comparison of the opalescence or turbidity produced by reagents, with a known turbidity, thus requiring an experienced worker. The Rachialbuminimètre of Sicard and Cantaloube[10] depends upon the measurement of the deposit of protein obtained by boiling cerebrospinal fluid with trichloracetic acid, and the tube must stand five hours before the height of the deposit may be read on the scale. Young and Bennet[11] add acidified alcohol to the fluid and then boil the mixture to precipitate the protein. The preparation is then transferred to vaccine tubes and centrifuged at high speed so that the height of the precipitate may be read. The vaccine tubes have a large upper portion, diminishing near the bottom to capillary dimensions. Centrifuging is difficult in that the precipitate tends to collect on the shoulder of the tube and makes it necessary to repeat the centrifuging one or more times. Moreover, the boiling, cooling, and transferring of the mixture from one tube to another, prove too time-consuming for a routine procedure.

*From the William Pepper Laboratory of Clinical Medicine, and the Department of Pediatrics, Hospital of the University of Pennsylvania.

The most accurate of all methods is the estimation of the protein content by first determining the total nitrogen by the Kjeldahl technic, then the non-protein nitrogen by the method of Folin and Wu. The difference of the two values, when multiplied by a factor, gives the amount of total protein in the sample. This procedure is complicated and obviously unsuitable for routine use.

A reagent containing 1.5 per cent of picric acid and 3.0 per cent of citric acid has long been recommended as a precipitant of protein.[12] The reagent and the specimen are placed in an Aufrecht tube[13] and centrifuged. The percentage of protein may be read directly from the calibrations at the bottom of the tube.

Fig. 1.

This method has several disadvantages. A larger volume of the specimens is required than is usually obtainable. The Aufrecht tube was originally intended for the estimation of albumin in urine and requires 4 c.c. of specimen fluid. Frequently, the amount of fluid safely removed from a patient for diagnostic purposes is barely sufficient for other laboratory work, which, in most instances, is more important than the protein estimation. The calibrations on the lower portion of the Aufrecht tube are so large that normal amounts of precipitate cannot be read.

Several features of the method recommend it. The test may be performed in three or four minutes of actual working time. It is extremely simple and requires little laboratory skill. This is important when we consider that most

spinal fluids are examined by interns, who are constantly changing services, and are not in the laboratory long enough to acquire skill equal to that of an experienced technician.

ALTERATIONS IN THE OLD TECHNIC

Having selected this method for development and standardization, it was found that none of the tubes on the market were suitable. A new tube was then designed, avoiding the bad features of previously used tubes.* This tube (Fig. 1) requires 1.5 c.c. of spinal fluid for the test, as we found that this was the amount of fluid usually remaining after the other laboratory procedures had been completed. A fine capillary bore was provided at the bottom of the tube to measure the small volume of precipitate obtained when using small quantities of spinal fluid. A similar but less suitable bore had been used in the vaccine tubes of Young and Bennet.† The abrupt narrowing of the small shoulder of the vaccine tubes was replaced by a small shoulder near the top of the tube, thus eliminating the collection of precipitate at this point. Below the shoulder, the bore is constant nearly to the bottom, where it gradually tapers to capillary dimensions. The tapered portion must be as short as possible because of the difficulty encountered by the manufacturers in calibrating accurately such a tube. The graduations represented by numerals indicate the divisions in cubic centimeters; each cubic centimeter is further subdivided into "units" having a value of $\frac{1}{250}$ c.c. The tubes are quite delicate and have to be well protected in centrifuging at high speed. A wooden holder was provided to hold the tube firmly and fit into the ordinary centrifuge bucket.

CENTRIFUGING

The only other modification in technic was the lengthening of the time of centrifuging. After the precipitate is thrown into the bottom of the tube, a definite amount of packing takes place. This is dependent upon the speed and length of time of centrifuging. In the original technic, the time is given as two minutes and the speed as 2,000 to 4,000 r. p. m. This was found to leave the precipitate so loosely packed that any variation in the speed or time would cause considerable error. By varying the speed and the time intervals, it was found that after fifteen minutes at 2,000 r. p. m., the amount of additional packing taking place, due to slight variations in speed or time, was negligible.

REAGENT

Dissolve 1.5 gm. of picric acid and 3.0 gm. of citric acid in 100 c.c. of distilled water. Solution is possible only when the flask is heated. After cooling, crystals will separate out and collect on the bottom of the flask. These are removed by filtering, and the filtrate is now ready for use. In pipetting the reagent, care must be observed to avoid transferring crystalline material to the test tube as obvious errors would then result.

*The authors wish to acknowledge the cooperation of the Kimball Glass Company of Vineland, N. J., in overcoming the technical difficulties encountered in the manufacture of this tube.

TECHNIC

The specimen of spinal fluid should be free of pellicle formation and therefore fresh fluids should be insisted upon. A cell count is of importance in estimating the effect of blood cells in the fluid.

The spinal fluid is placed in the tube with a capillary pipette, until the 1.5 c.c. level is reached, and the reagent added to the 3.0 c.c. level. After allowing several minutes for the diffusion of the two fluids, the tube is centrifuged at 2,000 r.p.m. for fifteen minutes. The height of the precipitate is then read off the calibrations of the tube. In instances where the amount of precipitate is so great that it reaches above the shoulder, near the top of the tube, the fluid should be diluted with equal parts of distilled water and another estimation made.

The tubes are readily cleansed by holding them under a running stream of water and at the same time inserting a capillary pipette connected with a suction tube. (Fig. 2.)

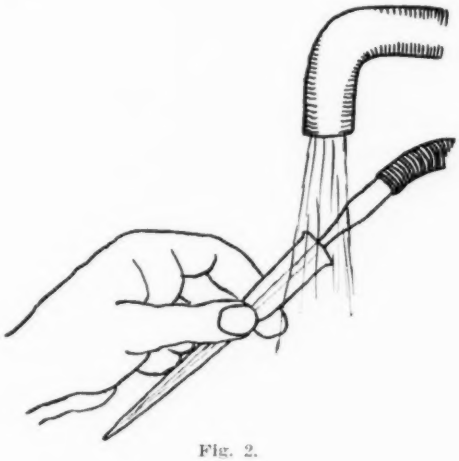

Fig. 2.

INTERPRETATION

This test is an estimation of the total precipitable protein and is not an estimation of the total protein. Picric acid may not precipitate all of the protein in the presence of an organic acid, but the portion that is precipitated bears a constant relationship to the total protein present (Fig. 3). This is an accurate index to the total amount of protein which may be readily determined by a simple calculation or by reference to Table I.

While the values given in Table I are approximate, they were obtained by plotting a curve based on estimations of 50 normal and pathologic fluids, and will be found sufficiently accurate for general purposes.

A normal reading is less than 45.0 mg. of protein or less than 1.5 units. The unit values are given in milligrams of protein per 100 c.c. The total protein was determined by estimating the total nitrogen by the Kjeldahl technic, the nonprotein nitrogen by the technic of Folin and Wu. The difference of

TABLE I

UNITS	MILLIGRAMS OF PROTEIN
0.1	16.0
0.2	17.5
0.3	19.0
0.4	21.0
0.5	23.0
0.6	25.0
0.7	27.0
0.8	29.0
0.9	31.0
1.0	33.0
2.0	50.0
3.0	67.0
4.0	85.0
5.0	102.0
6.0	120.0
7.0	138.0
8.0	155.0
9.0	175.0
10.0	192.0
11.0	208.0
12.0	227.0
13.0	245.0
14.0	262.0
15.0	280.0
16.0	297.0
17.0	315.0
18.0	332.0
19.0	350.0

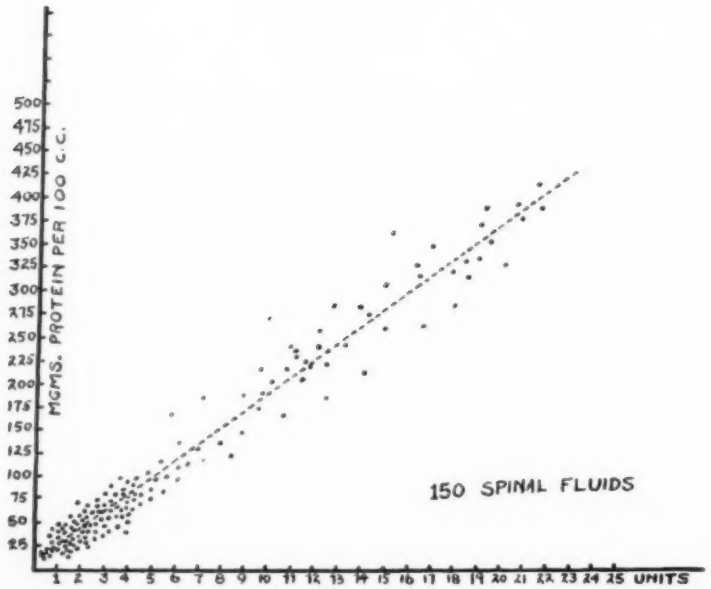

Fig. 3.

the two values represents the total protein nitrogen, which may be converted into the protein equivalent by multiplying by the usual factor.

ACCURACY OF TUBES

In testing the tubes for error, 15 tubes were used, each containing the same specimen of spinal fluid. The tubes were identical and as nearly accurate in calibration as is commercially possible. The greatest variation between any two tubes was 10 per cent. This happened with only one tube, the difference between the other tubes being less than 5 per cent. Since the normal variation of protein in spinal fluid is variously given as 17 to 50 mg. per 100 c.c. (a variation of 200 per cent), a percentage error of 5 per cent is negligible for practical routine purposes.

Numerous estimations, using the same tube and the same specimen of spinal fluid, revealed possible variations of plus or minus 15 per cent, although identical technic was used in each estimation. This supports our original contention that any method of estimating protein content by precipitation and centrifugation must be subject to an error of this extent. In spite of this error, the results are sufficiently accurate for many clinical purposes, as will be demonstrated below.

In attempting to diminish or eliminate this error, the following variations were studied:

1. The use of different reagents:
   a. 10 per cent trichloracetic acid.
   b. Sulphosalicylic acid.
   c. Mercuric chloride.
   d. Various combinations of these.
2. Altering the time of centrifugation.
3. Altering the speed of centrifugation.
4. Allowing the fluids to stand varying periods of time before centrifugation.
5. Varying the temperature of the spinal fluid and the reagent.
6. Incorporating gelatinous substances in the reagent:
   a. Agar agar.
   b. Sodium silicate.

In no case was the percentage of error reduced and in some instances it was definitely increased.

AGE OF SPECIMEN

Estimations made on a specimen of spinal fluid at two-day intervals revealed no alteration in the readings until the sixth day when the specimen became turbid. Readings of one unit had been consistently obtained until the turbidity appeared, when the reading increased to 1.5 units. The turbidity and increase in precipitable protein were due to bacterial growth. Where specimens must be preserved for such long intervals, the addition of a small amount of tricresol of toluol will prevent bacterial growth.

STABILITY OF REAGENT

A reagent which had been standing at room temperature for six months gave identical results on a specimen of spinal fluid as that obtained with a freshly prepared reagent.

EFFECT OF BLOOD CELLS IN THE SPECIMEN

The specimens of spinal fluid received by the laboratory are occasionally contaminated by blood due to trauma by the puncture needle. The amount of blood may be sufficient to render worthless the estimation of protein by this method, unless correction is made for the contamination. The contamination may consist of unchanged red blood cells and plasma protein, or altered red blood cells, undetectable on microscopic examination. In the latter instance, the proteins of the disintegrated cells are present, as well as the plasma proteins. Frequently, the contamination is due to old hemorrhage rather than trauma at the time of the tap.

The effect of unchanged red blood corpuscles and plasma protein was determined by adding fresh whole blood in varying quantities to a spinal fluid with known protein content. A protein estimation (chemical) and a red cell count were made on each sample of spinal fluid (Table II). It was thus found that a normal spinal fluid containing 9,250 red blood cells per c.mm. would have an increase in precipitable protein amounting to one unit, an increase of 100 per cent. Thus 10,000 red blood cells per c. mm. in a specimen of spinal fluid would increase the total protein about 32 mg. per 100 c.c. Therefore, a fluid containing 1,000 red blood cells per c.mm., would have an increase of protein of 3.2 mg., a figure below normal variation and hence one which may be ignored.

TABLE II

DESCRIPTION	CELLS	TUBE 1 (UNITS)	TUBE 2 (UNITS)	TUBE 3 (UNITS)	PANDY'S
Normal spinal fluid		1.0	0.95	0.87	Normal
Whole blood added	180,000	22.0	21.7	22.0	4-plus
Whole blood added	20,000	3.0	2.95	2.6	1-plus
Whole blood added	1,900	1.1	1.05	0.95	Normal
Whole blood added	160	1.0	0.95	0.82	Normal
Whole blood added	15-20	0.8	0.75	0.65	Normal
Washed red cells added	260,000	13.0	12.5	12.25	3-plus
Washed red cells added	22,000		2.0	1.9	1-plus
Washed red cells added	1,760		0.8	0.75	Normal
Washed red cells added	220		0.75	0.69	Normal
Serum representing	(260,000)		15.8	16.0	4-plus
Serum representing	(22,000)		2.9	2.8	2-plus
Serum representing	(1,760)		1.2	1.1	1-plus
Serum representing	(220)		1.2	1.1	Normal
Serum representing	(20-25)		1.05	0.95	Normal

The effect of altered red blood cells, undetectable on microscopic examination, may be determined by the Sahli hemoglobinometer or by some other colorimetric method. The approximate blood contamination is then calculated from the hemoglobin value.

BLOOD CELLS VERSUS SERUM AS CONTAMINANTS

To determine which constituent of the blood, the cells or the serum, contributed most to the protein increase in spinal fluid, washed red cells or plasma were added to specimens of spinal fluid with known protein content. Estimations indicated that cells alone account for 45.5 per cent of this increase, while the plasma accounts for 54.5 per cent. Approximately one-half of the protein increase is due to the serum albumin and serum globulin of the serum fraction, and one-half is contributed by the mechanical bulk of the cells.

CLINICAL APPLICATION

An analysis of the findings in 141 consecutive cases, in which routine specimens were sent to the Pepper Laboratory, will illustrate the grouping of results.

TABLE III

A. Nonpathologic	Readings 0-1.5 units	53	92.98%
B. Nonpathologic	Readings 1.6-2.0 units	1	1.75%
C. Nonpathologic	Readings over 2.1 units	3	5.26%
D. Pathologic	Readings of 1.5 units or less	10	11.92%
E. Pathologic	Readings 1.6-2.0 units	9	10.71%
F. Pathologic	Readings of 2.1 units or more	65	77.38%
	Total cases	141	

It is evident that in this group, 92.98 per cent of the "nonpathologic" specimens contained 1.5 units or less of protein precipitate, while of the "pathologic" specimens, 77.38 per cent contained over 2.0 units of protein. The cases in group A were miscellaneous and from their clinical diagnosis it was obvious that no process existed which would increase the protein content of the spinal fluid. The one case in group B had mastoiditis and gave a reading of 1.8. This may have been due to undiscovered extension of the process beyond the inner table of the mastoid. The three cases in group C were: pyelonephritis, with a reading of 2.3; hysteria, with a reading of 3.0; psychoneurosis, with a reading of 3.0. It is possible that an increase of protein was to be expected in all three cases, but they were included in the nonpathologic group because of the accepted views concerning the protein content of the spinal fluid in such cases.

Group D is unnecessarily large, since 6 cases were diagnosed as brain tumor and gave readings of 1.2, 1.3, 1.5, and 1.5. According to some observers, there is not an increase in the protein content of the spinal fluid in all such cases. Of the other 4 cases, encephalitis, with a reading of 0.8, and cortical cyst, with a reading of 1.4, may also be absolved of an increase in protein content, due to the pathologic process. Meningitis (cured), with a positive Wassermann, gave a reading of 0.4, and localized arachnoiditis, with a reading of 1.4, are also cases in which diagnosis may have been in error, or in which the protein content was not necessarily increased. Group E, with 9 cases, raises the suspicion that the diagnosis was in error.

Group F comprises the actual pathologic cases. Fourteen cases of brain tumor gave readings between 2.5 and 7.5, with one 23.0 and another 70.0

units. Thirteen cases of hemorrhage into the central nervous system, gave readings between 3.0 and 22.0 units. The findings in the meningitis group are presented in Table IV. Two cases of metastatic carcinoma of the brain gave readings of 42.0 and 2.4 units; two cases of carotid thrombosis, 2.2 and 2.7 units; traumatic myelitis, 21.0 units; alcoholic psychosis, 3.0 units; mul-

TABLE IV

TOTAL PROTEIN UNITS	W.B.C.
Meningococcic Meningitis	
5.0	
5.7	54
6.5	610
6.5	176
6.5	
7.5	1,100
7.5	13,270
7.5	690
8.0	955
8.0	86
8.0	101
11.0	3,200
14.0	10,640
18.0	6,800
18.0	5,000
18.0	
19.0	10,000
Tuberculous Meningitis	
2.4	107
2.7	83
3.0	340
4.0	38
5.0	79
5.5	56
14.0	92
Tuberculous Meningitis—Cont'd	
17.0	254
18.0	90
18.0	256
29.0	775
Unknown Meningitis	
11.9	285
6.75	157
3.0	109
Purulent Meningitis	
25.0	30,000
Syphilitic Meningitis	
3.0	
Meningitis Serosa	
1.5	
(Spinal fluid Wassermann negative)	
1.0	3
(Spinal fluid Wassermann negative)	
2.0	11
(Spinal fluid Wassermann negative)	
Focal Meningitis	
1.0	5
(Spinal fluid and blood Wassermann negative)	

TABLE V

Acute bulbar palsy	0.8
Bulbo-arachnoid hemorrhage	1.5
Brown-Sequard paralysis	0.9
Convulsions	1.6, 0.06
Concussion	1.5
Cerebral hemorrhage	9.0, 3.0, 6.0, 4.5, 22.0, 14.0
Chronic epidemic encephalitis	1.8
Cerebellar tumor	5.7
Diabetes, encephalitis	1.3
Deaf mute	1.4
Epilepsy	1.0, 1.2, 1.3, 0.45, 1.0
Epidemic encephalitis	21.0
Fractured skull	2.5
Headache	0.9
Hysteria	0.9, 1.0, 0.8
Hydrocephalus	1.0
Intracranial hemorrhage	6.0, 6.6, 5.3
Neurasthenia	1.1
Optic atrophy	1.0
Pansinusitis	1.5
Paraplegia	4.5
Post encephalitic syndrome	1.7
Sciatica	1.3, 1.5
Subarachnoid hemorrhage	5.1, 3.0
Spinal cord tumor	9.0
Unconsciousness	1.0
Uremia	1.0
Vertigo	1.2

tiple sclerosis, 4.0 units; serous meningitis 2.5 units; epidemic encephalitis, 21.0 units; cord tumor, 9.0 units; paraplegia, 4.5 units; atypical tic, 2.4 units.

In one case of pachymeningitis, the fluid from the ventricles and spinal canal gave normal readings, while fluid from the subarachnoid space gave readings as high as 60.0 units. Readings in units of other cases with the diagnosis as given the laboratory are shown in Table V.

This series is not sufficiently large to be conclusive, but the method in other hands may be productive of additional data which will form more definite groupings. Table IV presents the data obtained in the study of 37 cases of meningitis, while Table VI represents data from a study of 23 cases, diagnosed clinically as central nervous system syphilis. The agreement of the protein readings with the positive spinal fluid Wassermann in all but two cases is noteworthy. The two exceptions gave readings of 2.5 and 2.1 units.

Variations in reading account for an error of plus or minus 15 per cent, thus convincing us that any test employing precipitation, centrifugation, and direct reading, will not prove sufficiently accurate for purposes of research. We were unable to overcome this variation, but feel that this tube and method are valuable for ordinary clinical work. In support of this, we offer these analyses of cases, studied at the University Hospital, from 1927 to 1929. An additional support to this view may be found in the reliance placed upon the test by the Neuro-Surgical and Neurological Services at this hospital.

TABLE VI

CASE	CLINICAL DIAGNOSIS	SPINAL FLUID WASSERMANN	BLOOD WASSERMANN	W.B.C.	R.B.C.	TOTAL PROTEIN UNITS	REMARKS
1	C.N.S. syphilis Multiple sclerosis	Neg.	Neg.	27		1.0	Gold curve Syphilitic type
2	C.N.S. syphilis	Neg.				1.0	
3	C.N.S. syphilis	Neg.	Neg.	5		0.8	
4	C.N.S. syphilis	Neg.	Neg.	4		1.3	
5	C.N.S. syphilis	Neg.	Neg.	8		1.4	
6	C.N.S. syphilis	Neg.	Neg.	0		0.75	
7	C.N.S. syphilis	Neg.		9		2.1	
8	C.N.S. syphilis	Neg.		3		2.5	
9	C.N.S. syphilis	Neg.	Pos.			1.0	
10	C.N.S. syphilis	Neg.	Pos.	3		1.2	
11	C.N.S. syphilis	Neg.	Pos.	7		1.0	
12	C.N.S. syphilis	Pos.	Neg.			2.0	
13	C.N.S. syphilis ?	Pos. ?		48		2.1	
14	C.N.S. syphilis Tabes dorsalis	Pos.				2.5	
15	C.N.S. syphilis	Pos.		6		5.0	
16	C.N.S. syphilis	Pos.	Neg.	40	1,470	15.0	
17	C.N.S. syphilis	Pos.	Pos.	28		10.5	
18	C.N.S. syphilis Paresis	Pos.	Pos.			14.0	
19	C.N.S. syphilis	Pos.	Pos.	16		2.25	
20	C.N.S. syphilis	Pos. Neg. Pos.		4 9 9		6.8 2.0 3.3	Before treatment After treatment Later in course
21	Multiple sclerosis	Neg.		4		3.5	

CONCLUSIONS

1. Tests employing precipitation, centrifugation and direct reading for the quantitative estimation of protein in the cerebrospinal fluid are not sufficiently accurate for research purposes.

2. Homogeneous packing of precipitable protein during centrifugation is made possible by a new type of centrifuge tube.

3. Calibration of the centrifuge tube enables rapid estimation of the protein content of the specimen by direct reading.

4. The study of 141 consecutive pathologic and nonpathologic spinal fluids would indicate that the technic will prove of value in aiding or corroborating diagnosis.

5. The method is rapid, requires little laboratory skill and is sufficiently accurate for general clinical purposes.

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THE ELECTROMETRIC TITRATION OF URIC ACID*†

By Robert D. Barnard, Chicago, Ill.

DURING a recent investigation of the action of ultraviolet irradiation on uric acid[1] a need arose for a rapid method for the determination of uric acid in the presence of its decomposition products. The reduction of alkali ferricyanide by uric acid, as advocated by Flatow[2] was the method of choice, for it was found that this reduction was rapid, and that a slight excess of ferricyanide made an excellent visible end point, provided the quantity of fluid titrated was small and the concentration of ferricyanide employed in the titration sufficiently great (at least 5 millimolar). In lesser concentrations the excess of ferricyanide does not create an end point of clarity sufficient for exactitude so that recourse was had to potentiometric methods.

Electrometric titrations of uric acid were therefore secured. Since there was some desirability of extending the application of this titration to biologic fluids and since in the latter connection uric acid is usually separated as its silver, zinc or copper complex and these in turn are all soluble in alkali cyanide, the effects of the products of such solution on the titration were studied.

Solutions of uric acid in the presence of other substances mentioned, were titrated with potassium ferricyanide and the potentials after each increment were read against bright wire platinum electrodes. The chain in each case was arranged:

Pt | Urate | Saturated KCl | Hg2Cl2 | Hg

Stirring was effected by a glass nitrogen inlet tube which also served to diminish the oxygen tension of the solution.

The titrations were continued to reach the e_o value of the ferricyanide-ferrocyanide system, a procedure which forms a check on the end point of the oxidation of the urate.

RESULTS

Two equivalents are involved in the oxidation of alkali urate by ferricyanide. A typical titration curve is shown in Fig. 1. These results confirm the findings of Flatow.[2]

The presence of cyanide increases the negativity of the potentials obtained before complete oxidation of the urate, but does not affect the number of equivalents involved in this oxidation (Fig. 2).

From Table I it may be seen that the titration is valid in the presence of zinc, copper, and silver cyanides which result when the urate complexes of

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TABLE I

TITRATIONS OF LITHIUM URATE IN THE PRESENCE OF VARIOUS SUBSTANCES

PRESENT (MM./L)	FOUND	SOLUTION IN:
0.85	0.86	1 M NaCn – 1 M NaOH
0.85	0.85	50% H_2SO_4
0.85	0.87	20% $Zn(CN)_2$
0.85	0.85	5% $Cu(CN)_2$
8.81	8.76	Recovered as silver precipitate dissolved in 2 Molar cyanide

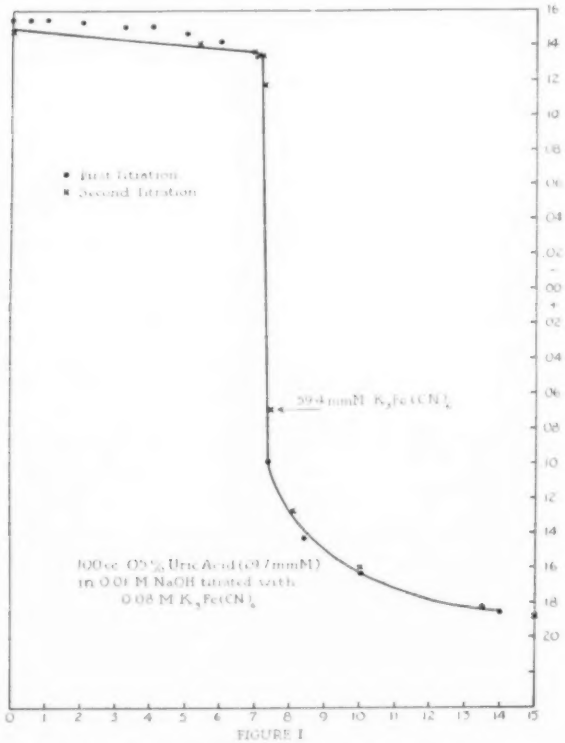

FIGURE 1

Fig. 1.—Potentiometric oxidation titration curve of alkali urate.
100 c.c. of 0.05 per cent uric acid (29.7 mmM) in 0.01 M NaOH titrated with 0.08 M $K_3Fe(CN)_6$.
Ordinates represent potentials against bright platinum on the saturated KCl – calomel scale, abcissae show the number of c.c. of 0.08 M $K_3Fe(CN)_6$ utilized in the titration.

these metals are brought into solution with alkali cyanide. It is interesting to note that solutions of uric acid in strong sulphuric acid are amenable to the ferricyanide titration, though it is necessary to take the visible end point in this case. The amount of uric acid shown to be present in urine by the Folin-Wu[3] method was recovered as well as quantities of pure uric acid which had been added to urines of known content (Table II).

It was found that freshly prepared solutions of sodium cyanide gave increased uric acid values, though in themselves the reduction of ferricyanide was negligible. Solutions which had been aged forty-five days were therefore used and gave theoretical recovery on known uric acid solutions. Later it was found that when the cyanide was dissolved in 1 Molar sodium hydrox-

TABLE II

RECOVERY OF URIC ACID FROM URINES OF KNOWN CONTENT*

PRESENT	ADDED	RECOVERED	ADDED RECOVERED	PER CENT RECOVERY
12.76*	8.81	21.57	8.81	100.0
6.46*	0.85	7.26	0.80	94.1
6.46	1.70	8.11	1.65	97.1
6.46	2.55	9.98	2.52	98.8
16.40*	0.0	15.88		96.4
12.44*	0.0	12.32		99.0

All concentrations are expressed as millimols per liter

*By the Folin-Wu method.

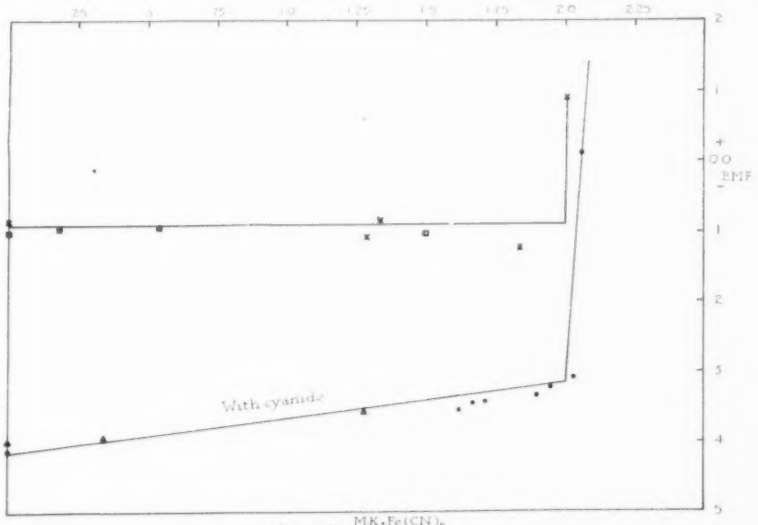

Fig. 2.—The effect of cyanide on the irreversible oxidation-reduction system.
Uric acid – 2= allantoin. A, shows representative potentials obtained in a solution of alkali urate during the course of a titration with ferricyanide. B, shows potentials observed in the same system after addition of cyanide. Ordinates give potentials on the saturated KCl – calomel scale, and the abcissae gives the ratio of the concentration of ferricyanide in Mols to the concentration of urate in Mols. X, Experiment 1A. □, Experiment 2A. △, Experiment 1B. O, Experiment 2B.

ide it was fit for use immediately. A sample of sodium cyanide* gave high results immediately after preparation, after aging for from one to ten weeks and in 1 Molar NaOH solution. In the titration of solutions containing this sample of cyanide, after the theoretical end point had been reached there was no cessation of the reduction of ferricyanide and on continued addition, a disturbing brownish color would develop. In working with more concentrated solutions of uric acid than those usually employed (10 per cent), it developed that a color reaction took place in solutions containing, as well, ferricyanide, and cyanide. Whereas, ferricyanide, when added to solutions of cyanide containing reducing substances such as phenols or glucose, will develop a dirty brownish color, in the case of uric acid, this color is a bright orange which appears to be distinctive and if it develops in solutions subjected to the analytical procedure obscures a visible end point and indicates, potentiometrically, that the reduction of ferricyanide is being caused through

*Central Scientific Co.

some side reaction. When the cyanide employed is made to a concentration of 1 Molar in sodium hydroxide, this color reaction and the undesirable reactions which it apparently portends, do not develop.

Since the titration of uric acid by ferricyanide was found to be applicable to the silver urate recovered from urine by the isolation procedure of Folin and Wu and since it was considerably more convenient than the latter, inasmuch as no standard or color comparison is required, a procedure has been developed which is being used routinely at the Berwyn Hospital and which is being found to check excellently with the colorimeter procedure on all urines thus far analyzed. This procedure for urine has been developed with the collaboration of Dr. Theodore Heller of Berwyn Hospital to whom the author acknowledges his indebtedness.

PROCEDURE FOR URINE

Solutions Required.—1. A solution containing 5 per cent of silver lactate in 5 per cent lactic acid (Folin and Wu).

2. Twenty millimolar potassium ferricyanide. There are 6.584 gm. of Merck's Reagent $K_3Fe(CN)_6$ dissolved in water and the volume made up to one liter. This solution should be kept in the dark. One solution thus prepared has kept its titer for over six months.

3. One molar sodium cyanide in one molar sodium hydroxide. Dissolve 4 gm. of NaCN in 100 c.c. of normal NaOH solution.

METHOD

One c.c. of urine is pipetted to a 15 c.c. conical centrifuge tube, diluted with distilled water to a volume of approximately 6 c.c. and 8 c.c. of silver lactate solution added. After the precipitate has "clumped" it is rapidly centrifuged for two minutes, the supernatant poured off as completely as possible and the precipitate suspended in one c.c. of distilled water by means of a fine glass rod. The alkali hydroxide-cyanide solution is then added from a medicine dropper disturbing the precipitate after the addition of each drop until it forms a clear and almost colorless solution. This will require from two to six drops. The preliminary suspension of the precipitate and gradual addition of the cyanide avoids the formation of black silver oxide which may occur if these points are not observed. To the solution in the centrifuge tube the standardized ferricyanide solution is added from a one c.c. microburette until the addition of one drop causes persistence of the lemon yellow color, or, if the titration is followed electrometrically until there is a sharp potentiometric increment.

CALCULATIONS

Each c.c. of ferricyanide is the equivalent of 10 millimillimols of uric acid or one c.c. of ferricyanide is equal to 1.68 milligrams of uric acid.

SUMMARY

The practicability of following the titration of uric acid by ferricyanide potentiometrically is demonstrated and a method for the determination of uric acid, based on this principle, is described.

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A SLIDE COMPARATOR FOR P_H DETERMINATIONS*

By W. A. Taylor, Ph.D., Baltimore, Md.

AS A RESULT of the long and varied experience of their entire organization with P_H control equipment and methods, W. A. Taylor & Company has developed the new slide comparator. This instrument is unique in design and construction. Being molded from Bakelite, it combines the maximum of durability, precision, convenience, and simplicity.

The slide comparator consists of two principal parts, the slide and the base. The slide is a Bakelite case 10 inches long, 2¾ inches high, and ⅝ inch thick. It contains 17 vertical holes and 17 horizontal slots which pass through the exact centers of the holes. In these holes are placed the 9 color standards for any given indicator and 8 ampules of distilled water. All these ampules are held in place by a lid which is screwed on the top of the slide.

The base consists of two parts. The lower part contains a slot in which the slide may be moved back and forth, two holes containing vials of indicator solution, five holes containing test tubes, and a closed compartment for a ground glass plate. Horizontal slots run through the three central holes in the base holding the test tubes, these slots corresponding exactly with any three of the slots in the slide. The ground glass is contained in a closed compartment directly behind the three slots in the base. The upper part of the base serves as a cover for the vials and test tubes when the set is not in use. It is fastened to the lower part by means of spring catches.

In making determinations, the top is removed from the base, three of the test tubes are filled to the mark (5 c.c.) with the sample to be tested and placed in the three holes back of the slots in the base. To the central tube 0.5 c.c. of the indicator solution is added by means of the pipette and nipple and the contents are thoroughly mixed. The slide containing the color standards is now placed in position on the base and, holding the instrument toward a window or other source of daylight, the slide is moved back and forth in front of the test samples until a color match is obtained. The P_H is then read off directly from the values on the front of the slide.

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Being molded from Bakelite the slide comparator is unaffected by water, acids or alkalis. It therefore will not warp or come apart. Molding also gives it a machined precision that is impossible with wood or sheet metal.

Since the color standards are enclosed in a Bakelite case, there are no loose standards to be inserted and removed in making P_H determinations. Thus all chance of loss or breakage from handling individual standards is eliminated. All color standards and test tubes are 11.5 mm. outside diameter.

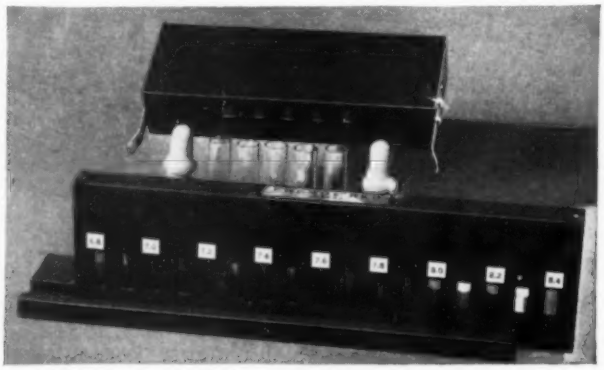

Fig. 1.

Fig. 2.

This small equipment makes the set small and compact and therefore readily portable. Of far greater practical importance, however, is the fact that the resulting thinness of layer of material being tested enables one to make accurate determinations on far more highly colored and turbid materials, without dilution, than is possible with larger tubes.

Determinations are made simply by sliding the color standards in front of the test sample until a color match is obtained.

Slide comparators are supplied to cover the P_H range 0.2-13.6. Each comparator covers a range of 1.6 P_H units, the standards being in intervals of 0.2 P_H.

LONG RANGE SLIDE COMPARATOR

The long range slide comparator was designed to meet the need of those workers who require a portable set covering a wide P_H range. This set is made in five models to cover the range of any 3, 4, 5, 6 or 7 indicators respectively between the limits of P_H 0.2 and 13.6.

Each long range slide comparator contains one complete slide comparator 2 to 6 extra color standard slides; vials of the corresponding indicator solutions, with pipettes and nipples; and a supply of 5 c.c. test tubes. This equipment is contained in a portable case 11 inches long, 9½ inches wide and 4½ inches high.

872 Linden Avenue.

A CLINICAL METHOD FOR THE DETERMINATION OF SERUM PROTEINS*

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THE increasing knowledge of the rôle of the proteins in maintaining the osmotic equilibrium of the organism renders a method for their quick and accurate determination in various body fluids of increasing importance. The previous methods for their estimation require a large expenditure of time and are essentially complicated laboratory procedures. Among these may be mentioned: 1, the physical, including refractometry, viscosimetry, and any combination of these; 2, the colorimetric method of Wu; and 3, the most widely used, some application of the Kjeldahl digestion with titration or Nesslerization of the formed ammonia. We have developed a method on an entirely different principle which transforms the determination of the protein content of sera or exudates and transudates into a most simple clinical procedure, and available for regular routine estimations with sufficient accuracy for clinical purposes. However the same principle can be applied in larger laboratories for the very rapid determination of proteins electrometrically. We estimate the amount of protein by the buffer power of the solution containing it on a solution of known hydrogen-ion concentration.

Since the work of Loeb it has become generally known that the proteins are best pictured as amphoteric electrolytes of the formula $R<^{COOH}_{NH_2}$ where R stands for any organic complex. Whether the ionization will be as an acid or a base is entirely dependent on the hydrogen-ion concentration of the solution. In solutions more acid than the iso-electric point of the protein we may

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disregard the ionization of the COOH group and assume for all practical purposes that the protein functions as a substituted amine-RNH_2. Hence it will combine with the available acid and remove from the solution a certain amount of hydrogen ion. If we work at sufficiently acid solutions we cut down the ionization of the carbonic and other weak acids of the blood so that they may be disregarded and the protein remains the dominant factor in regulating the hydrogen-ion concentration of the solution.

The Colorimetric Clinical Determination of Protein.—Five-tenths c.c. of blood serum is diluted with an equal volume of distilled water and 0.25 c.c. of N/10 HCl and two drops of methyl red are added. This solution contains

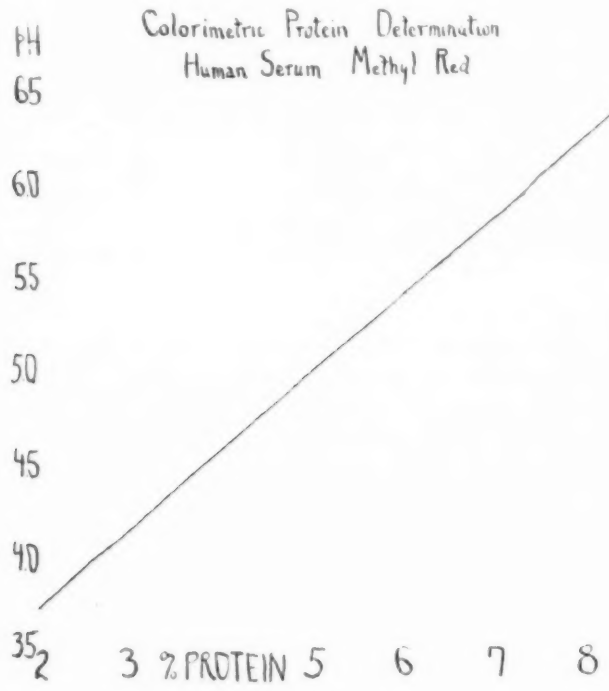

Chart 1.

0.02 gm. in 60 c.c. alcohol plus 40 c.c. water. A color appears instantly. Yellow, over 6 per cent protein; yellow orange, 5 per cent; pink orange, 4 per cent; pink, 3 per cent; red, below 3 per cent. This furnishes results sufficiently accurate for most clinical purposes. If more accurate results should be required a set of acetate buffers between the range of P_H 4 and P_H 6 with methyl red may be obtained and the protein concentration determined from Chart 1. The Hellige hydrogen-ion comparator which has a special disc for this range with permanent glass standards has been found excellent. Actually the simple color scheme given above will be found sufficient for determining whether the protein in the blood is low or normal.

Determination of the Protein Content of Exudates and Transudates.—To one c.c. of the fluid 0.25 c.c. of N/10 HCl are added and two drops of methyl

red. The colors obtained range from the red of less than 1 per cent protein or below through orange to the yellow of 3 per cent above, thus enabling an extremely rapid determination of the protein content and hence the classification into exudates and transudates.

Approximately 100 sera were examined and parallel determinations made by the Kjeldahl method. It was found that the error was within 0.5 gr. of protein per 100 c.c. of serum. In three cases of nephrosis with edema a distinctly red color was obtained and the protein digestion method showed a protein content of 3.1, 2.9, and 3.4 per cent respectively. The turbidity of the

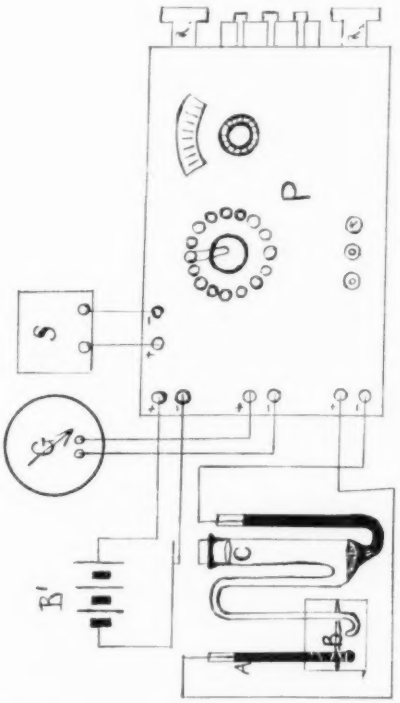

Fig. 1.—A quinhydrone electrode *A* dips down into a beaker containing the solution to be measured into which a few milligrams of quinhydrone have been stirred. Connecting with this by a saturated KCl solution is a calomel reference electrode *C*. *B* is the storage battery, *S*, a standard Weston cell, and *P*, a potentiometer.

serum due to the high lipoid content did not interfere. One case of suspected slight edema showed an orange red color equivalent to the buffer of 5.2 and was shown to have a protein content of 4.9 per cent. Certain of the sera which gave an orange yellow color, P_H 5.5, contained about 6 per cent protein and were to be classed as low normals. In cases of the cachexia of cancer and nutritional disturbance of infancy an orange pink color, P_H 5.0, was obtained, and the sera showed a protein content of 4.6 and 4.8 per cent respectively.

In order to get accurate standards for the above clinical method parallel electrometric determinations were carried out. We worked at a hydrogen-ion

concentration sufficiently high to cut down the ionization of all the weakly ionized acids of the blood and leave the protein as the dominant factor in buffering the solution. An additional advantage of working within this range is that it enables us to work with quinhydrone electrodes. The arrangement of the apparatus is shown in Fig. 1. The P_H of a solution measured with the quinhydrone electrode and a saturated calomel reference electrode when the temperature is 25° is given by the equation

$$\frac{0.453 - V}{0.0591} = \log \frac{1}{\left[\overset{+}{H}\right]} = pH$$

where V is the potential difference between the electrodes. Chart 2 gives the titration curve of serum with N/10 HCl. Chart 3 shows the titration

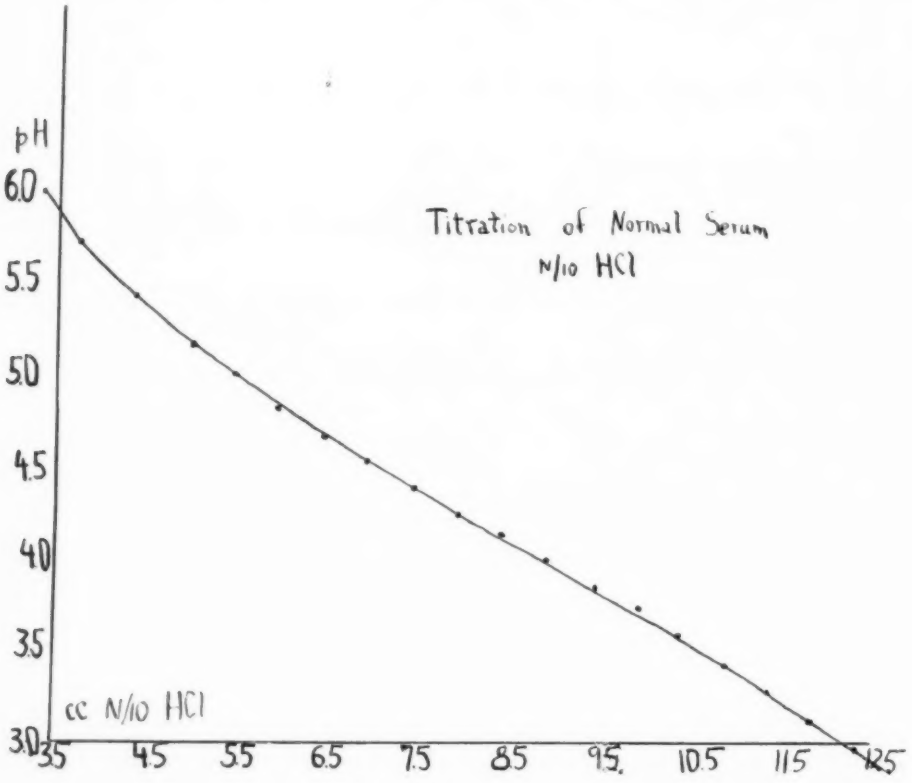

Chart 2.

curves of various pathologic sera and exudates, and it is apparent that within certain ranges the amount of acid required to cause a unit change in P_H is proportional to the concentration of the protein. In Chart 4 various dilutions of sera were titrated with N/10 HCl and at a certain point taken arbitrarily (actually the point best suited to the colorimetric estimation of the proteins) an ordinate was drawn. The points where this ordinate cut the various titration curves were used as the new ordinates, and as abscissae the protein con-

TABLE I

P_H OF VARIOUS DILUTIONS OF SERUM MADE UP TO 10 C.C. ON THE ADDITION OF 2.5 C.C. N/10 HCl

SERUM, C.C.	PROTEIN, GR./100 C.C.	E.M.F., VOLTS	P_H	P_H (GRAPH)
5.0	7.23	0.1624	4.93	5.03
4.5	6.51	0.1835	4.57	4.52
4.0	5.78	0.1932	4.40	4.44
3.5	5.06	0.2065	4.18	4.12
3.0	4.34	0.2272	3.81	3.86
2.5	3.62	0.2483	3.47	3.47
2.0	2.89	0.2747	3.02	3.07
1.5	2.17	0.2891	2.78	2.77
1.0	1.45	0.3104	2.42	2.43
0.5	0.72	0.3409	1.90	2.00
0.0		0.3458	1.78	

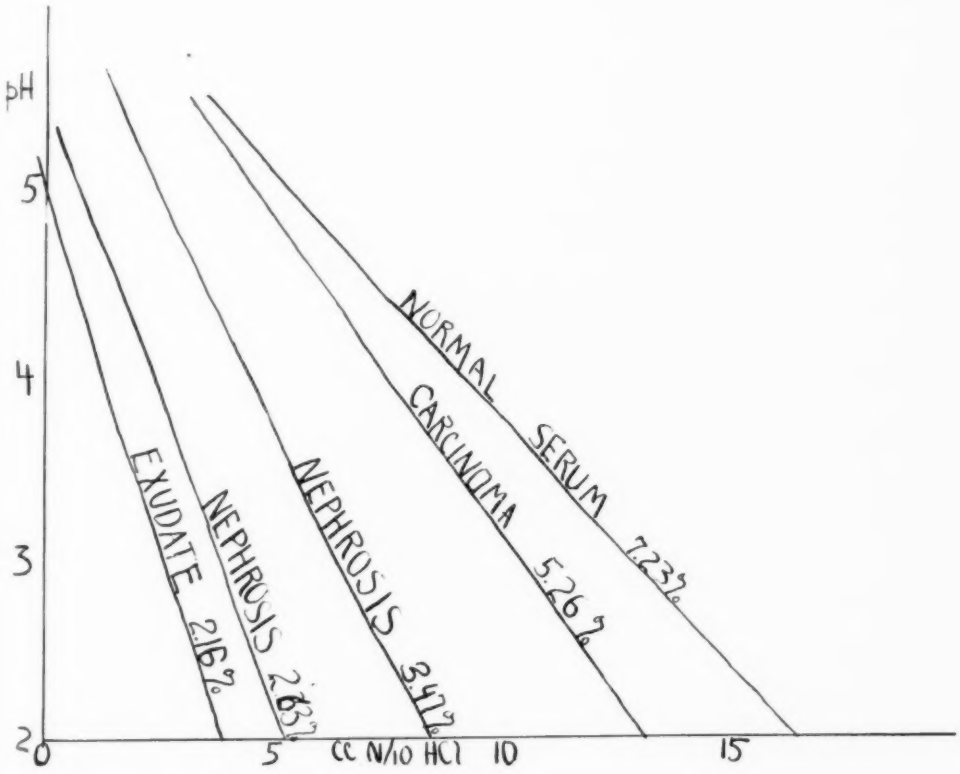

Chart 3.

centrations of the solutions were used. The line joining these curves was the graph of the change in hydrogen-ion concentration with variations in the protein content. In the actual electrometric determinations of unknown sera 2 c.c. of serum were diluted with an equal volume of water and 1 c.c. of N/10 HCl was added. The P_H of this solution was determined and the protein concentration read from Chart 4.

Sera were diluted to various protein concentrations at a fixed volume of 10 c.c. and 2.5 c.c. of N/10 HCl added. The P_H of these solutions should be

identical with those of Chart 4. Table I shows this to be the case within the limits of experimental error.

SUMMARY

An extremely simple method is given for the estimation of the protein content of either blood serum or exudates which gives results sufficiently accurate for clinical purposes. The same principle, that the shift in hydrogen-ion concentration in an acid solution on the addition of a protein is proportional to the concentration of the protein itself, is used as a method of electrometric protein determination.

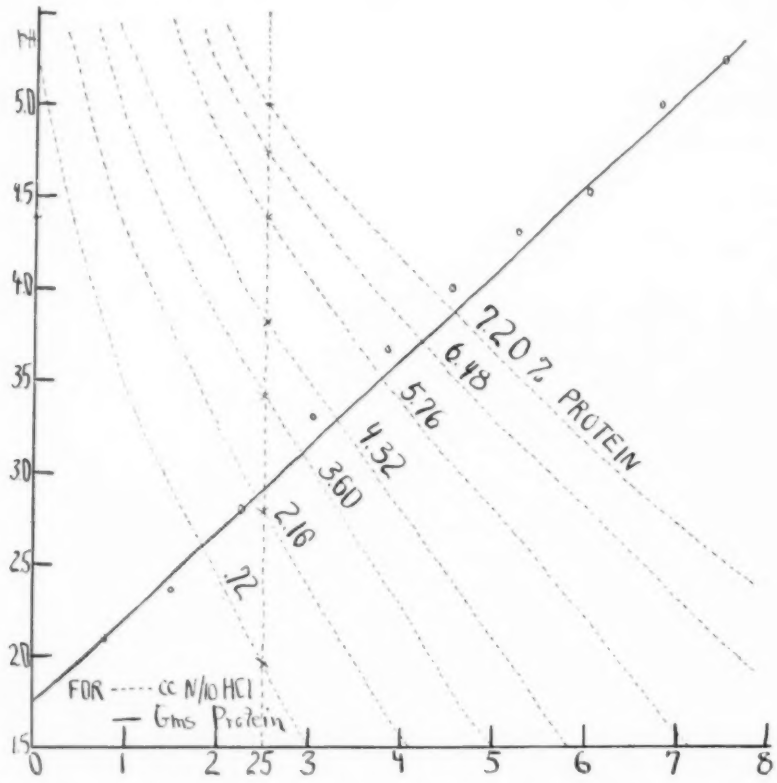

Chart 4.—Curve of various concentrations of serum titrated with N/10 HCl. The unbroken line joins the points where the various protein concentrations cut the 2.5 ordinate and indicate the relation between Pᴎ and protein concentration. Circles indicate actual observation of various dilutions of a serum originally containing 7.5 per cent protein.

NOMOGRAMS FOR METABOLISM ESTIMATIONS*

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THE use of apparatus of the oxygen consumption type for estimating the metabolism of patients is rapidly increasing. The purpose of this article is to make available a method of calculating the final result, which is at once easier, less time consuming, and well within the limits of accuracy of the apparatus used at present, and also to point out a means of simplifying the calculations for metabolism estimations by gas analysis methods.

Chart 1 is a modification of Bruen's[1] nomogram for the estimation of normal basal metabolism but has been adapted, recalculated and rearranged for greater convenience and ease in manipulation. First, place a ruler on the person's weight in the proper age group on the male or female side of the chart, and direct it perpendicularly inward until it crosses the height line. The point of crossing lies between two diagonal lines indicating c.c. O_2 or calories. Estimate the distance from the cross to the lower diagonal in tenths of the total distance. Each interval between diagonals equals 10 c.c. per minute or 2.9 calories per hour. One-tenth the interval is, therefore, equal to 1 c.c. O_2 or approximately 0.3 calories per hour. The number of tenths multiplied by these values is to be added to the values found on the diagonal below the point of crossing. This gives directly the basal caloric requirement (DuBois standard) in total calories per hour or the corresponding c.c. of oxygen per minute at a fixed respiratory quotient of 0.82.

In the apparatus most commonly used in this country, Krogh, Benedict-Roth, Sanborn and McKesson machines, the oxygen consumption is recorded graphically by change in position of an index on a moving drum in a given time. A line drawn through the expiration points produces a diagonal, the height of which is measured at the points of commencement and end of the test. The difference between the two heights is proportional to the c.c. O_2 or calories expended, but the correction for temperature, pressure and water vapour has not been made.

Chart 3 indicates the percentage correction required for a given temperature and pressure when the air is 80 per cent saturated with water vapour, the approximate condition of the air in these machines. Lay the rule on the barometric pressure and find the point of crossing the correct temperature line. This point lies between diagonals. The figures on the nearest diagonal are sufficiently accurate for our purposes but there is no difficulty in estimating to 0.5 per cent. To one accustomed to the use of charts this is much easier than searching in tables for the correct figure. Next, lay the ruler on Chart 2 over the figure corresponding to the observed value for c.c. O_2 or

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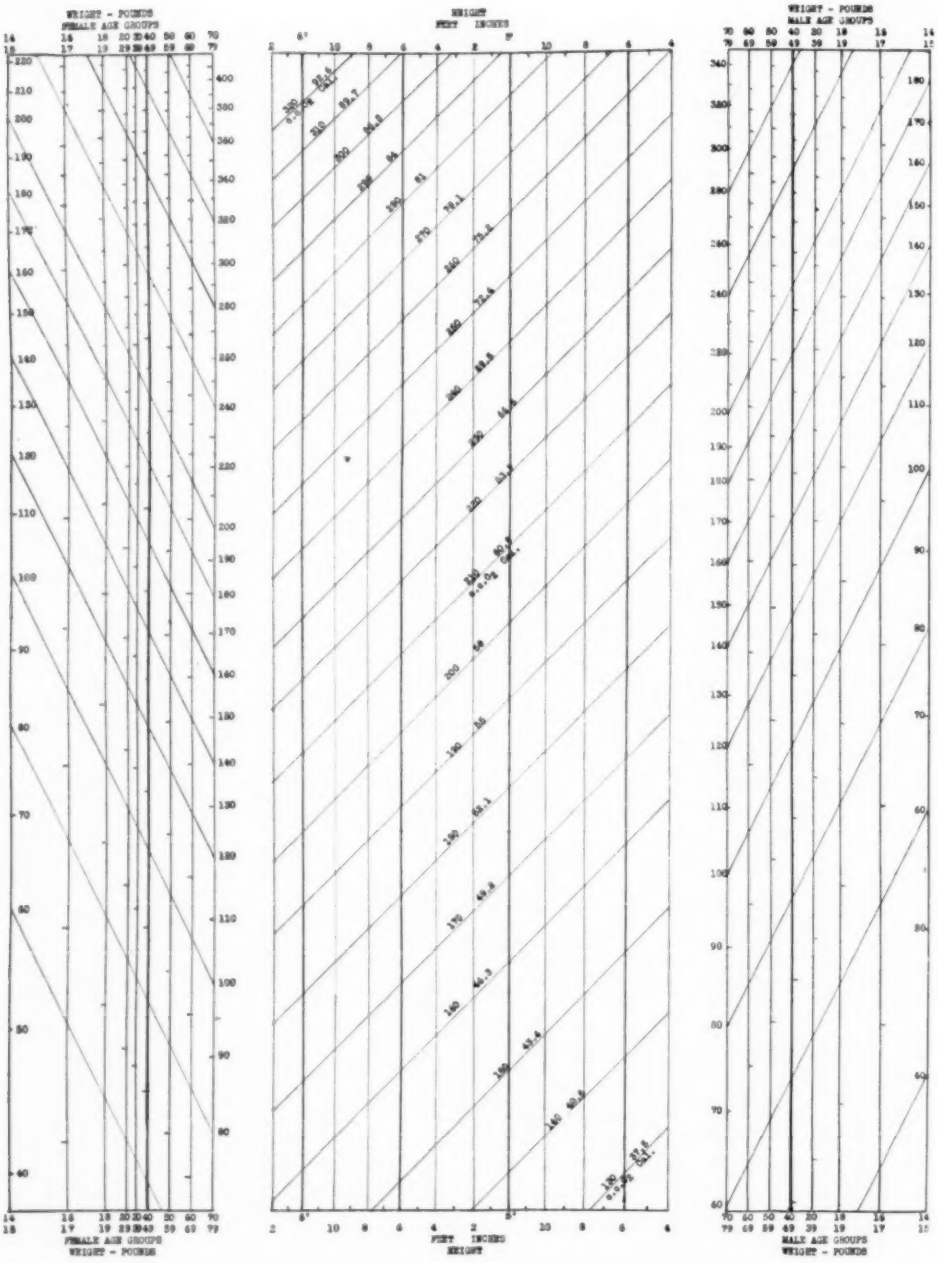
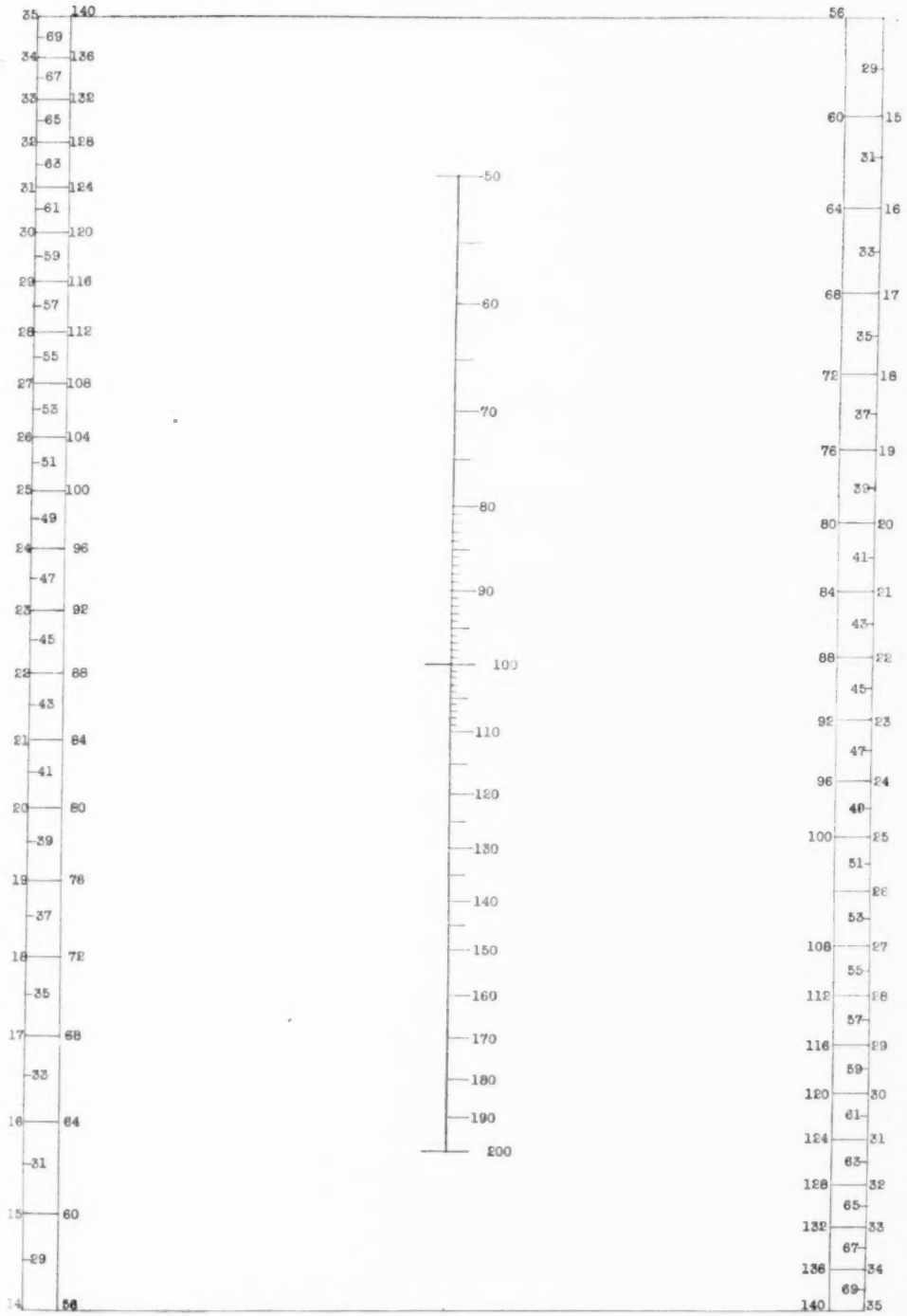

WEIGHT - POUNDS
FEMALE AGE GROUPS
FEMALE AGE GROUPS
WEIGHT - POUNDS
HEIGHT
FEET INCHES
FEET INCHES
HEIGHT
WEIGHT - POUNDS
MALE AGE GROUPS
MALE AGE GROUPS
WEIGHT - POUNDS

Chart 1.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128 32
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132 33
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136 34
69
140 35

Chart 2.

calories used (left side) running it through the center line at the pressure temperature correction just obtained. The point of crossing the right side lines is the actual number of c.c. O_2 used, or calories expended, reduced to 0° C. and 760 mm. for temperature pressure and water vapour. Maintaining the ruled line on this reduced value, place the other end on the normal value (obtained from Chart 1) on the left side of the nomogram. Read off the center line the metabolic rate in *percentage of the normal*. The whole operation requires fifteen seconds.

Application to the Sanborn Graphic Apparatus.—On the Sanborn apparatus the recording paper has vertical ruled time lines and horizontally ruled volume lines, but a correction of the values has been introduced so that at 21° C. and 760 mm. pressure the lines read the correct values for 0° C. and

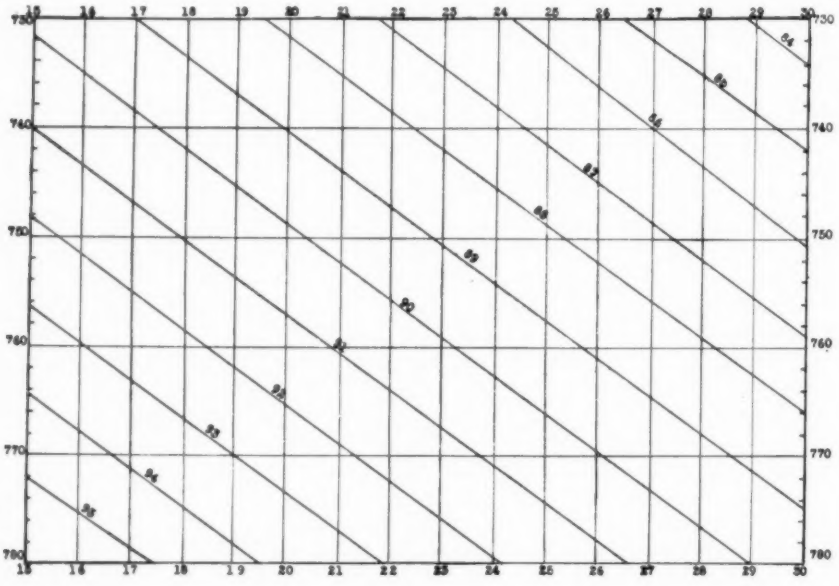

Chart 3.

760 mm. Hg pressure, free of water vapour. For other temperatures and pressures a correction obtained from tables must be added or subtracted. If the ruled recording paper is used (and there are some advantages in retaining it), Chart 4 is used instead of Chart 3 to make the suitable correction. A plain white sheet of typewriter paper may be used instead, the line drawn through the expiration points and two perpendicular lines erected ten and one-half inches apart, and the distance from the base to the point of intersection with the diagonal measured with a rule graduated in one-tenth inches.* Each inch equals 100 c.c. O_2 uncorrected, and it is easy to estimate to one-fiftieth of an inch or 2 c.c. The correction value is then found on Chart 3 (not Chart 4) and the calculation completed as above. A sharp pencil with hard lead is suggested for ruling the lines for the sake of definiteness, but the error in

*If desired, a base line and perpendiculars may be drawn on film and laid over the chart.

measurement due to the pencil used is not nearly so great as the possible error in fitting the diagonal through the expiration points.

In actual use, charts such as those illustrated here are mounted back to back between two pieces of celluloid obtained by washing the emulsion from discarded x-ray films. The ruler is made by drawing a fine line with a knife across another film. To avoid parallax, it is applied as closely as possible, ruled side down, to the nomogram.

By the more elaborate methods of calculation recommended for these machines, a result is obtained which is mathematically speaking more exact than that derived from the charts. Were this desirable, greater accuracy could be attained by increasing the size of the nomograms. This, however,

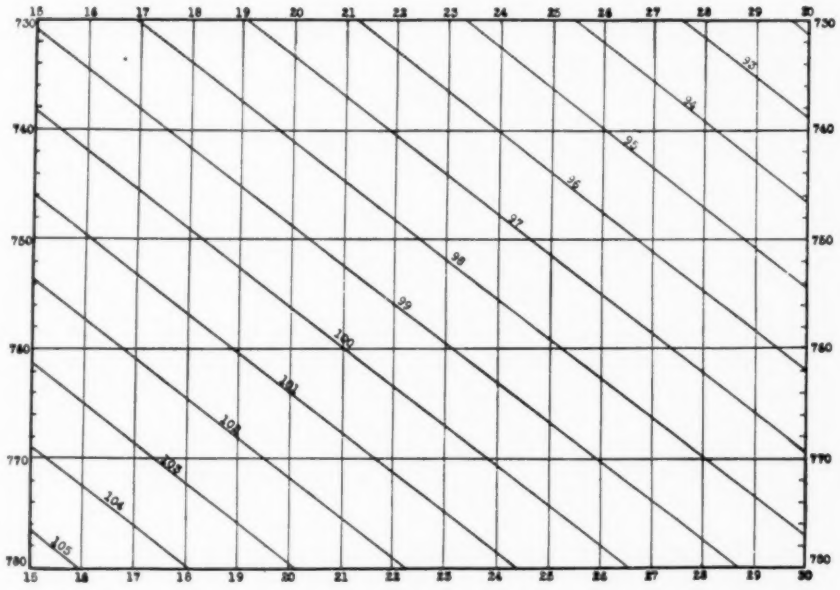

Chart 4.

is unnecessary since errors inherent in the oxygen consumption method itself make such accuracy superfluous. In point of fact also, such methods of calculation are of no greater clinical value since differences of less than one per cent, or sometimes even 5 per cent, can be ignored.

Applications to Metabolism Estimations by Gas Analysis.—It should be clearly understood that, no matter how accurately the determination of the actual oxygen absorbed in a given time may be, it is only a close approximation to the value obtained under similar conditions at another time. Further, it should be realized that the limits of normality are rather wide and that the DuBois surface area formula does not perfectly represent the actual surface area of any individual. While the gas analyses themselves are sufficiently accurate to be regarded as determinations, the metabolism test as a whole must be regarded as an estimation. For certain purposes it may be

desirable to make this as close as possible to a determination. For most purposes, however, considerable time in calculation may be saved by the application of nomograms such as these.

From Chart 1 the total normal metabolism in calories per hour is obtainable, and this represents a slight saving of time. Since the number of cubic centimeters of oxygen is calculated on a fixed respiratory quotient of 0.82, the oxygen consumption figures are necessarily incorrect for all other respiratory quotients. Since using these figures represents a considerable saving of labour it is worth while making a slight correction when using Chart 2 to compensate for this. For greater exactness one may use a right-angled T drawn on a film to ensure perpendicularity of the height and weight lines.

Chart 3, being calculated for only 80 per cent saturation with water vapour, is not suitable for gas analysis calculations. A similar chart on squared paper, 10 lines to the inch, is used. Millimeters of mercury are one-tenth inch apart; one-half degree C. three-tenths inches apart. An additional diagonal drawn halfway between the present ones assists in dividing the distance which the crossing point lies below a main diagonal into tenths, thus giving a three-figure correction. Since Chart 2, unless drawn very large, does not permit of estimations to less than one-fifth of one per cent, estimations in tenths is quite sufficient.

When the true respiratory quotient differs from 0.82, the correction to be made in using Chart 2 is to add 0.01 to the pressure temperature correction for each 0.04 above 0.82; or subtract a similar amount when it falls at 0.78. The accumulated error in comparison with five-place logarithms and four-place temperature pressure correction tables, may reach one-half per cent, but is usually less.

In actual practice the number of cubic centimeters of O_2 corresponding to the calculated normal metabolism is obtained from Chart 1. The indicated volume of gas collected is multiplied* by the gasometer factor, then by the percentage of CO_2 obtained by analysis.† From this figure the number of c.c. of CO_2 excreted per minute is obtained. Lay the film ruler on this figure in the *right-hand column* and run the line through the center line at the true respiratory quotient figure. The left-hand columns show the number of c.c. O_2 used per minute, but this figure is as yet unreduced. To reduce to dry air at 0°C. and 760 mm., make use of the suitable factor obtained from a chart made as described above or from tables, again using Chart 2 (this time from left to right) for reducing, but correcting for the difference between true respiratory quotient and 0.82 and obtaining the percentage of the normal as described for the oxygen consumption method. The result should be marked in some way to indicate that the short method of calculation has been used.

It may be desirable to emphasize one or two points. The film ruler is an actual necessity and close contact with the chart insures a high degree of accuracy. Considerable error is introduced by using an ordinary rule. It may sometimes be more convenient to use somewhat larger charts. For

*A ten-inch slide rule with magnifying cursor is sufficiently accurate.
†Minus the CO_2 percentage in outside air.

Charts 1, 3 and 4 photographic enlargements can be made without serious error. Chart 2, however, must be redrawn exactly to scale as slight errors due to expansion or shrinkage of the paper materially affect its accuracy. The scale used is a logarithmic one.

SUMMARY AND CONCLUSION

A method for calculating metabolic rates by the use of suitable nomograms is presented. This materially decreases the time required for this calculation without a significant sacrifice of accuracy. It is applicable to most of the methods in common use.

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AN ECONOMICAL CAGE AND A WATER PIPETTE FOR FEEDING EXPERIMENTS WITH MICE*†

By Franklin C. Bing, New Haven, Conn.

THE mouse has been used as an experimental animal in laboratory investigations beginning, at least, with the physiologic researches of Robert Boyle in 1670. Some of the problems in genetics, immunology, and medical research that have been solved with mice were mentioned by Daniel[1] in 1912. More examples could be furnished now, particularly from the field of nutrition, where the small food consumption coupled with the rapid growth rate of these animals has been of distinct advantage. In certain nutritional studies with mice that have extended over the past two years we have developed a few points in the technic of handling the animals that may be of value to other investigators. The present account describes an inexpensive and satisfactory cage suitable for feeding experiments with mice, and a simple and efficient drinking pipette for recording the water intakes.

The cage is shown in Figs. 1 and 2. It is based upon one that Dr. E. C. MacDowell of Cold Spring Harbor has used in some of his work. We have found this cage to be entirely satisfactory for one mouse; it allows ample room for exercise, and animals grow normally and live their usual span of life with no more space than that provided. The construction of the cage is clear from the illustrations. The container proper consists of an ordinary tinned bread-baking pan that is procurable in a standard size, approximately 5 × 9 inches long and 3 inches high. Similar pans may be obtained in aluminum or in glass, but at higher prices. The false bottom and the top piece are cut from

*From the Laboratory of Physiological Chemistry, Yale University, New Haven, Connecticut.

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†The data in this paper are taken from a dissertation presented by Franklin C. Bing in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Yale University, 1930.

galvanized wire screen of ¼ inch mesh. The lightest weight screen, known in some localities as "hardware cloth" is satisfactory, although that illustrated is of somewhat heavier material. The light weight material can be obtained in convenient rolls 1 foot in width. A paper towel or a piece of blotting paper is fitted in the bottom of the pan. Over this is placed the false bottom, which may have a hole cut in it for the food receptacle, as shown. For the food

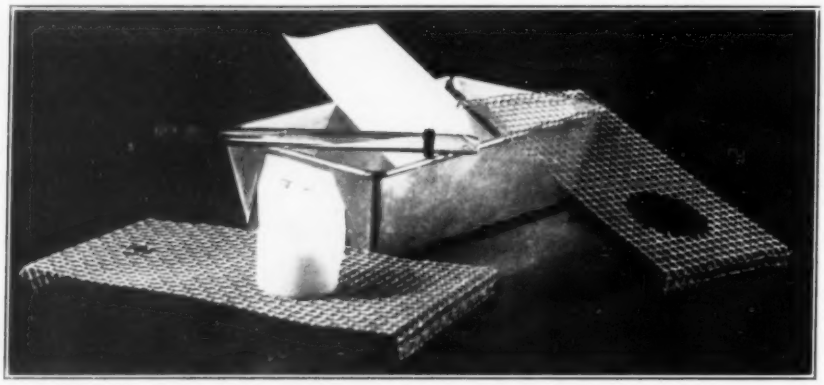

Fig. 1.—The parts of the mouse cage are shown, including the food cup and drinking pipette.

Fig. 2.—The photograph shows the assembled mouse cage.

cup, the small porcelain dish used in the Yale Laboratory of Physiological Chemistry, or a one-ounce porcelain ointment jar may be used. If the latter is supplied with the usual aluminum screw-cap one may adapt the device used by Parsons[2] to prevent the tendency of mice to scratch out their food. The aluminum top is prepared by cutting in it a circular opening about 2 cm. in diameter; the size of the opening must be smaller than the inside diameter of the jar and yet large enough to allow the mouse free access to the con-

tents. Over the food is placed a circular piece of $\frac{1}{4}$ inch mesh wire screen, which must be slightly smaller than the inside diameter of the jar, in order to drop down as the food is consumed, and somewhat larger than the opening in the cover of the jar, in order to prevent the mouse from pulling it out. The presence of the screen guard effectively prevents mice from digging out their food, but does not interfere with their food consumption.

The top piece of wire screen is simply bent to fit as a cover to the pan and the spring of the wire holds it fast. It should have a hole cut in it for the insertion of the water tube. The ends of the tinned pans have a fold and,

Fig. 3.—The drawing shows the water pipette and the beaded rod. For dimensions, see text.

following Dr. MacDowell's practice, a card bearing the number of the experiment and other data is placed in the space so provided.

The advantages of the false bottom cage, in making coprophagy difficult, have been pointed out by Steenbock, Sell and Nelson,[3] and have been subjected to rigorous experimental proof by Smith, Cowgill and Croll.[4] The present cage is perfectly suitable for Vitamin B or Vitamin G assays with mice. In addition, the MacDowell cage offers several advantages over the other types of cages that have been used for individual mice. It is cheap and serviceable. Material for fifty cages may be bought for less than ten dollars. Pans and wire screen parts made two years ago are still in usable condition. The assembled cages take up very little room and, after being dismantled, may be stacked away in a small space. The solid sides apparently protect

the mice from draughts, and although some of our animals have been observed over extended periods, none has developed "snuffles" or other respiratory infections while in these cages.

Other types of mouse cages may be required for special purposes, and it may be of interest to mention briefly some that have been described in the literature. Joseph Priestley,[5] in 1774, published in considerable detail his procedure for caging and maintaining the house mice which he caught and used in his work on the physiologic effects of gases. Among other points he stressed the necessity of cleanliness and a uniform temperature for animals, and he described a "receptacle for keeping mice" that contained a removable and readily cleaned false bottom.* Von Struve[6] described in 1849 a cage, containing clay and moss in order to imitate natural conditions, which he had used as early as 1811 for studying the life history of the mouse. In 1891 Socin[7] utilized a glass funnel, a porcelain plate and some silk waste to make a cage for use in an investigation where possible contamination with metals had to be avoided. Similar cages made of glass or of glass and porcelain have been described by Hall,[8] Bertrand and Benzon[9] and, most recently, by Hubbell and Mendel.[10] Cages for stock animals have been devised by Daniel,[1] Robertson[11] and others. Mitchell[12] has described a cage that is suitable for feeding experiments with large or adult mice; Beard[13] has employed a cage identical with the type ordinarily used for rats in the Yale Laboratory of Physiological Chemistry; and Dawburn[14] has made use of a clay dish in some of his feeding experiments.

In conjunction with the baking-pan cage we have employed a drinking pipette that enables one to record the fluid intake of experimental animals, if desired. It consists of a glass tube, similar to that used by Daniel and others. A convenient size can be made from a piece of glass tubing of 10 mm. bore and about 15 cm. long. One end is constricted to a diameter of about 4 mm. and the other expanded slightly to hold a No. 00 rubber stopper. The feature of the present water tube is the inclusion of the beaded glass rod shown in Fig. 3. This is made from 3 mm. stock, and is fashioned so that it rests snugly upon the shoulder at the bottom of the tube and acts, in a sense, like a valve. The presence of the rod increases the efficiency of the tube considerably. The water is less apt to drip, there is practically no loss by evaporation, and clean water is available to small mice at all times.

By using a graduated piece of tubing for the water pipette one may obtain satisfactory records of the quantitative intake of each mouse. Professor L. B. Mendel has also pointed out that one might administer a limited number of drugs in aqueous solution and, by means of the drinking pipette, obtain an accurate record of the intake of substances that the mouse will not eat voluntarily.

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*A reproduction of the wood-cut frontispiece of Priestley's book, *Experiments and Observations on Different Kinds of Air*, that includes a drawing of this prototype of the modern false bottom cages is given in Bayliss, W. M., *Principles of General Physiology*, ed. 4, p. 603, 1927.

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THE STAINING OF BLOOD-GROUPING SERA FOR PRESERVATION AND IDENTIFICATION*

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CONTAMINATION of hemagglutinating sera by bacteria may be a source of error in blood grouping tests for the following reasons:

1. The hemagglutinins can be decomposed by bacteria with a resulting loss of the agglutinating power of the serum.

2. The development of various bacteria in the serum may give rise to new products which in turn may cause pseudoagglutination of the red cells (Prati[1]).

3. Contaminated serum often contains flocculi of agglutinated bacteria which interfere with the readings of the test.

In routine hospital work where blood grouping is performed very frequently and often urgently, it is difficult to maintain the sterility of grouping sera. In order to protect the sera from bacterial contaminations, the addition of various antiseptics, such as chloroform, phenol, trieresol, quinosol, boric acid, sodium fluoride and glycerine was tried. But, as it has been noted by Lattes,[2] under the influence of these preservatives the sera become turbid and form precipitates. He, therefore, advises the use of sterile sera without recourse to antiseptics.

For more than two years I have been preserving sera by addition of dyes, such as brilliant green, acriflafine, gentian-violet and mercurochrome, the bac-

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tericidal and bacteriostatic properties of which are well established. The concentration of the dyes in the serum was brought up to 1:2500—1:5000.

The advisability of staining the sera may be questioned a priori, since it is well known that dye solutions by themselves can agglutinate red cells. But, as I have found in collaboration with O. Hornick,[3] even small quantities of serum are sufficient to protect red blood cells from agglutination by dyes. Therefore the staining of the sera does not introduce any new agglutinating factor. Numerous experiments on various sera have shown that the addition of dyes in no way affects the specificity of the hemagglutinins. The group of the stained sera remained unchanged and their agglutinating titer was practically the same as that of the unstained control portion which was kept in the ice box under sterile precautions. Stained sera kept at room temperature for many months did not lose their transparency. As a rule no development of bacteria took place in these sera although they were handled without any precautions of sterility and sometimes were kept in open tubes for weeks. The technic for grouping with the stained sera does not differ from the usual method. The clumps of the agglutinated red cells stand out very distinctly on the stained background.

The staining of sera by dyes has one additional advantage. By choosing special colors for various groups we can easily mark the sera and avoid confusion which may occur due to errors in labeling, etc. After several trials I adopted the following procedure: To each c.c. of serum group II was added 0.01 c.c. of 1 per cent aqueous solution of neutral acriflavine and 0.01 c.c. of 0.5 per cent aqueous solution of basic fuchsin. The combination of these two dyes was selected for the reason that acriflavine is a more powerful antiseptic than fuchsin and the latter stains the serum more strikingly. To each c.c. of serum group III was added 0.02 c.c. of 1 per cent aqueous solution of brilliant green. Thus we marked our group II sera red, and our group III sera green. This method of marking, I believe, is simpler and provides a greater safeguard against confusion than the method of packing sera in glass tubes of different colors suggested by the Health Committee of League of Nations.[4]

CONCLUSIONS

The addition of dyes protects the hemagglutinating sera from bacterial contamination without affecting their specificity and potency. By selecting dyes of different colors, it is easy to mark sera of various groups.

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A COMPARATIVE STUDY OF 350 SPINAL FLUID EXAMINATIONS WITH THE TAKATA-ARA TEST*

By S. T. Walton, M.S., D.V.M., Detroit, Mich.

THE mercuric chloride-fuchsin test for the detection of abnormal properties in spinal fluid specimens was devised in 1925 by the two Japanese, Takata and Ara. Their claim for the test was that it gave a typical reaction in cases of bacterial meningitis and in syphilis; the former being a color change in the reagent solution, from blue to pink or red, and the second a precipitation. This was described as being specific for syphilitic conditions.

So was Lange's colloidal gold test put forth as a means of differentiating spinal fluids of patients with meningitis, and for separating those of the different types of syphilitic infection. The gum mastic and benzoin tests for spinal fluids also have their supporters who believe that there is nothing like their own pet procedures for revealing the true story of a nervous system involvement.

Solutions of shellac have been prepared to be used as the reagent in tests on spinal fluids, and the scientist von Thurzo[1] propounded a method using gum guaiac solution colored with napthol green and brilliant fuchsin. Greenfield and Stern[2] reported favorable results with this test, placing its value on an equal basis with Lange's colloidal gold test in the laboratory diagnosis of neurosyphilis.

But of all these methods none are infallible, and all must be criticized for irregularities of one kind or another. Since none of them have any true antigenic or biologic affinity for the sensitive components of pathologic spinal fluids, all should be looked upon with some degree of skepticism, and not too much trust placed in a lone reaction without other corroborative evidence of the fluid possessing abnormal properties.

It is to be remembered that in all these colloidal solutions any degree of stability which they possess is due to the fact that certain optimum conditions, with no wide limitations, of electric charge, hydrogen-ion concentration and freedom of the dispersing solution from foreign particles of any kind, are maintained.

This being the case, and since we have recourse to no better procedures for definitely determining the causative factors which produce abnormalities of the nervous system, it seems wise to give consideration to any method which gives promise of proving helpful in the diagnosis of the difficultly solvable cases that are often met with in the study of nervous and mental diseases.

The Takata-Ara test has been variously experimented with by European and American investigators. Some agree and some disagree with the origi-

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nators of the test as to its practical value as a laboratory diagnostic measure.

Blum,[3] Cameron and McCulloch,[4] and Monias[5] have reported results with the test which compare favorably with other laboratory methods and clinical findings. Karnosh and King,[6] and Kasahara and Konishi[7] disagree with these and believe that the test is not what the authors claimed for it in the way of specificity.

I have made a comparative study of 350 examinations with the Takata-Ara test, using Lange's colloidal gold and the gum mastic tests as a basis for comparison, along with the Kolmer-Wassermann test.

Technic: The technic employed is the modification reported by Monias in which he substituted N/10 sodium hydroxide for the 10 per cent sodium carbonate solution used by the authors. As observed by Monias and verified here, this substitution brings out the color change more vividly in the color reaction, producing a greater contrast which is more easily read.

Another slight change in technic is the use of a 0.4 per cent sodium chloride solution instead of 0.3 per cent as originally advocated. This was done as a matter of convenience, using the same salt solution as is used for making dilutions in the Lange test. A few experiments showed that there was no difference in the reaction if a greater salt concentration was used, up to 1 per cent.

Necessary reagents:

0.4 per cent sodium chloride solution (made up in ordinary distilled water—one distillation).

N/10 sodium hydroxide solution.

0.5 per cent mercuric chloride solution ($HgCl_2$).

0.02 per cent aqueous solution of Diamant-fuchsin (G. Grubler & Co.).

For the test, set up a row of five Wassermann tubes. Put 1 c.c. of the salt solution in all except the first. Add 1 c.c. of spinal fluid to the first and second tubes. Mix the solutions in the second and transfer 1 c.c. to the third. Continue this procedure to the fifth tube. Discard 1 c.c. from the fifth. Now add one drop of the N/10 sodium hydroxide solution to all tubes and shake. This may be added with an ordinary 1 c.c. serologic pipette and will measure from 0.04 to 0.05 c.c. in volume. Finally add 0.3 c.c. of the reagent to each tube. The reagent is a mixture of equal parts of the bichloride and fuchsin solutions which is prepared immediately before addition to the tests. The separate solutions may be kept indefinitely without deterioration. Shake the tubes and leave at room temperature for twelve to eighteen hours (overnight).

Interpretations: The color of the solution in the tubes when set up is a very deep purple which is almost blue. The change of the color toward a pink or red is usually indicative of bacterial infection and is recorded in three degrees as follows: 1, reddish violet ; 2, motley pink; 3, clear, distinct pink or rose colored. A fluffy precipitate, which remains suspended in the solution, may or may not appear concomitant with the color change. This is not easily confused with the heavy precipitates which occur in syphilis and settle out, leaving the supernatant fluid clear and colorless if precipitation is complete, or grayish-blue of varying intensities in case of incomplete precipitation.

Five degrees of precipitation are recorded, 1, 2, 3, 4, 5, according to the observer's judgment as to the amount of precipitate present, basing the comparison on the appearance of a tube which shows complete precipitation; number 5 indicating complete precipitation.

This method of recording each type of reaction with numerals instead of plus signs is a variant from the author's, and is substituted in order to have the report correspond to the manner of recording other results. This seems to be advantageous in that a more comprehensive summary of results is obtained at a glance.

Positive reactions occur in case of diseases of the nervous system other than syphilis and the true forms of meningitis just as positive reactions also are given with the gold and mastic tests in these other conditions. But like the latter also, they are seldom strongly positive and are of little significance in the absence of other corroborative information.

No definite conclusions as to the exact merits or demerits of the test will be given, but a table of comparative tests with the older laboratory methods on fluids from patients suffering with a number of various conditions will show the reaction in each case.

DISCUSSION

Meningitis.—Thirty-four cases. Two specimens gave strongly positive color reactions that were negative Lange and mastic. One negative Takata-Ara (T.A.) gave a strongly positive Lange and mastic, the gold curve being of the tabetic type, and one negative with a very low gold curve. Three gave a precipitate with no color change. One of these was positive Lange and mastic and one negative. The other was positive gold and negative mastic.

Syphilis.—Fifty-eight cases. Six out of the 58 specimens from cases diagnosed as central nervous system syphilis, including general paralysis, tabes and dementia paralytica, gave a positive T.A. with negative Lange and mastic, and all but one had a positive blood Wassermann; that was a case of primary syphilis. Another case with no history available gave similar results; both the blood and spinal fluid Wassermann were strongly positive. An eighth gave positive T.A., Lange and Wassermann with negative mastic.

Five cases showed a negative T.A. with positive gold, mastic and Wassermann.

Organic Disorders.—Fifty-two cases. Of these there were 19 specimens which gave divergent results. Eleven were positive T.A. and negative gold and mastic, while five gave the reverse action. In two other instances the T.A. agreed with the gold, and with the mastic in one.

One of these cases had a diagnosis of psychosis with a question of syphilis and a 4-plus blood Wassermann. Otherwise they were not considered syphilitic nor as having general bacterial infection of the central nervous system.

In the 203 negative specimens all three tests agreed in their results and agreed with other findings so did not receive further consideration.

TABLE I

COMPARISON OF TAKATA-ARA TEST WITH LANGE'S COLLOIDAL GOLD AND MASTIC ON SPINAL FLUID EXAMINATIONS

CASE	BLOOD WASS.	WASS. (SP. FL.)	LANGE	MASTIC	TAKATA-ARA	CONDITIONS
1	0	0	0000000000	0000000000	C.*30000 P. -----	Meningococcus meningitis
2	No record	No record	0000011000	3443211000	C. 10000 P. -----	Meningococcus meningitis
3	No record	No record	1223432200	5555432100	C. ----- P. -----	Meningococcus meningitis
4	0	0	0000121000	2110000000	C. 21000 P. -----	Meningococcus meningitis
5	0	0	0000111223	5555554322	C. 31000 P. -----	Meningococcus meningitis
6	0	0	0000000000	0000000000	C. 30000 P. -----	Meningococcus meningitis
7	No record	0	0111133200	4543211000	C. 11000 P. -----	Meningococcus meningitis
8	No record	No record	0000000011	1234433211	C. 31000 P. -----	Meningococcus meningitis
9	0	0	3221110000	0000000000	S. ----- P. -----	Meningococcus meningitis
10	0	0	2111100000	0000000000	C. ----- P. 20000	Meningococcus meningitis
11	0	0	0000011100	3445443210	C. 30000 P. -----	Epidemic meningitis
12	0	0	0000001111	3555433210	C. 20000 P. -----	Epidemic meningitis
13	No record	0	0000012211	1221100000	C. 31000 P. -----	Epidemic meningitis
14	No record	0	0000011100	1110000000	C. 10000 P. -----	Epidemic meningitis
15	0	0	0001223311	5554322100	C. 31000 P. -----	Epidemic meningitis
16	0	0	0000000123	5555555432	C. 33100 P. -----	Epidemic meningitis
17	0	0	2100122211	1122100000	C. 21000 P. -----	Epidemic meningitis
18	0	No record	0000001111	4555543210	C. 31000 P. -----	Epidemic meningitis
19	No record	0	1111222333	5555544210	C. 31000 P. -----	Epidemic meningitis
20	++++	0	0000123344	5555555542	C. 33100 P. -----	Epidemic meningitis
21	No record	0	0000011100	4321000000	C. ----- P. 42000	Epidemic meningitis
22	0	0	0000022100	1110000000	C. 10000 P. -----	Tuberculous meningitis
23	0	0	0000122111	2221000000	C. 21100 P. -----	Tuberculous meningitis
24	0	0	0000012211	0111000000	C. 32000 P. -----	Tuberculous meningitis
25	0	0	0000012100	1221100000	C. 30000 P. -----	Tuberculous meningitis
26	No record	0	0000111000	1110000000	C. 20000 P. -----	Tuberculous meningitis
27	No record	0	0000000000	0000000000	C. ----- P. 30000	Tuberculous meningitis
28	++++	++++	0000132100	2211000000	C. 10000 P. -----	Syphilitic meningitis
29	++	++++	1111233322	3432211000	C. 11000 P. -----	Syphilitic meningitis
30	No record	Ac.	0000011221	455543210	C. 32200 P. -----	Purulent meningitis
31	0	0	0000112110	4444421000	C. 10000 P. -----	Purulent meningitis

*C, color change
P, precipitation

TABLE I—CONT'D

CASE	BLOOD WASS.	WASS. (SP. FL.)	LANGE	MASTIC	TAKATA-ARA	CONDITIONS
32	No record	0	0000012211	5555554222100	C. 21000 P. _____	Hemorrhagic pachymeningitis (Staphylococcus)
33	No record	0	0000121000	3221000000	C. 10000 P. _____	Hemorrhagic pachymeningitis (Staphylococcus)
34	No record	0	0000122100	5544322100	C. 10000 P. _____	Hemorrhagic pachymeningitis (Staphylococcus)
35	No record	0	0000112100	0000000000	C. _____ P. 20000	Arteriosclerosis, general and cerebral
36	0	0	5543222210	1221000000	C. 20000 P. 02000	Arteriosclerosis, general and cerebral
37	No record	0	0000001111	1244432100	C. 30000 P. _____	Arteriosclerosis of central nervous system; brain abscess
38	No record	0	4445554100	5553221000	C. _____ P. 40000	Brain abscess
39	No record	0	0000000000	0000000000	C. _____ P. 30000	Brain abscess
40	0	0	0000111000	3210000000	C. 10000 P. _____	Brain tumor
41	0	0	1122344200	5554432100	C. _____ P. 55000	Brain tumor
42	0	0	0000021100	2211100000	C. _____ P. 52000	Brain tumor
43	0	0	5554432100	4322211000	C. _____ P. 50000	Brain tumor
44	0	0	0000000000	0000000000	C. _____ P. 20000	Brain tumor (possible)
45	No record	0	1112332100	3221100000	C. _____ P. 50000	Spinal cord tumor
46	0	0	0000111000	1110000000	C. _____ P. 40000	Spinal cord tumor
47	0	0	0012232100	4433210000	C. _____ P. _____	Brain tumor ? Syphilis (history)
48	0	0	1112321000	4221000000	C. _____ P. 20000	Chronic encephalitis
49	0	0	0001110000	0000000000	C. _____ P. 40000	Chronic encephalitis
50	0	0	1122210000	0000000000	C. _____ P. _____	Chronic encephalitis
51	No record	0	0000000000	0000000000	C. _____ P. 40000	Cephalalgia
52	++++	0	2222210000	2211100000	C. _____ P. _____	Psychosis (syphilis?)
53	No record	0	4422210000	2110000000	C. _____ P. 40000	Psychosis (senile)
54	++++	++++	5555555410	5554321000	C. 20000 P. 54400	Psychoneurosis (syphilitic history)
55	0	0	0000111000	1110000000	C. P. 20000	Dementia praecox
56	0	0	0000000000	0000000000	C. 10000 P. 00000	Dementia praecox
57	0	0	5200122100	5555200000	C. 33331 P. _____	Dementia praecox
58	0	0	0000011111	1110000000	C. 20000 P. _____	Dementia praecox
59	0	0	0000000000	0000000000	C. 10000 P. _____	Dementia praecox
60	No record	0	1244543332	5555554431	C. 30000 P. 55000	Dementia praecox
61	No record	0	4444454321	5555543211	C. _____ P. 54000	Dementia praecox
62	++++	++++	1111244210	5555443321	C. 10000 P. 54000	Dementia paralytica
63	0	++++	4444554310	5543210000	C. _____ P. _____	Dementia paralytica

TABLE I—CONT'D

CASE	BLOOD WASS.	WASS. (SP. FL.)	LANGE	MASTIC	TAKATA-ARA	CONDITIONS
64	++++	++++	5555555542	5555542100	C. _____ P. 55430	Dementia paralytica
65	++++	++++	5555542110	5555432210	C. _____ P. 54000	Dementia paralytica
66	++++	++++	5555555410	5554221000	C. _____ P. 54000	Dementia paralytica
67	++++	++++	1223452100	5554332100	C. _____ P. 40000	Dementia paralytica
68	++++	++++	5555555420	5555432100	C. 10000 P. 55000	Syphilis
69	+++	0	0000111010	22221100000	C. 10000 P. 40000	Syphilis
70	++	0	0000000000	0000000000	C. ____ P. 40000	Syphilis
71	++++	++	5411110000	2211000000	C. _____ P. 50000	Syphilis
72	0	0	0012221000	1100000000	C. _____ P. 30000	Syphilis
73	++	++++	2444432100	4221000000	C. ____ P. 42000	Syphilis
74	++++	0	0112221000	2221100000	C. ____ P. 40000	Syphilis
75	++++	++++	1111100000	2211100000	C. _____ P. 40000	Syphilis
76	++++	++++	1111221100	4211000000	C. ____ P. 50000	Syphilis
77	++++	0	0000000000	0000000000	C. _____ P. 40000	Syphilis
78	+++	0	0000000000	0000000000	C. _____ P. 30000	Syphilis
79	+++	0	1111121000	0000000000	C. _____ P. 30000	Syphilis
80	0	0	0000000000	0000000000	C. _____ P. 30000	Syphilis (primary)
81	++++	+++	0011100000	1110000000	C. _____ P. _____	Syphilis
82	++++	0	0000000000	0000000000	C. _____ P. 40000	Syphilis
83	++	++++	2222321000	3211000000	C. _____ P. 52000	Syphilis (tertiary)
84	0	++++	5541231000	4321100000	C. ____ P. 40000	Syphilis
85	++++	++++	4222211000	2110000000	C. _____ P. 50000	Syphilis
86	0	++++	4322221000	3211100000	C. _____ P. 50000	Syphilis (history)
87	No record	0	4422210000	2110000000	C. _____ P. 40000	Syphilis (history)
88	++++	++++	3333321000	2110000000	C. _____ P. 40000	Syphilis of central nervous system
89	++++	++++	1111232210	4442210000	C. _____ P. _____	Syphilis of central nervous system
90	++++	++++	2444431100	2211000000	C. _____ P. 40000	Syphilis of central nervous system
91	++++	++++	2344443100	5554322100	C. _____ P. 52000	Syphilis of central nervous system
92	++++	++++	5555554221	5555433100	C. _____ P. 54000	Syphilis of central nervous system
93	No record	++++	1112341000	2211100000	C. _____ P. _____	Syphilis of central nervous system
94	No record	++++	1112231000	4421100000	C. _____ P. 40000	Syphilis of central nervous system
95	++++	++++	4443221000	4443321000	C. _____ P. 40000	Syphilis of central nervous system

TABLE I—CONT'D

CASE	BLOOD WASS.	WASS. (SP. FL.)	LANGE	MASTIC	TAKATA-ARA	CONDITIONS
96	No record	++++	55554432100	5544432210	C. _____ P. 50000	Syphilis of central nervous system
97	++++	++++	1112341000	55553221100	C. _____ P. 50000	Syphilis of central nervous system
98	++++	++++	00001110000	11100000000	C. _____ P. 20000	Syphilis of central nervous system
99	++++	++++	1122321000	22110000000	C. _____ P. 40000	Syphilis of central nervous system
100	++++	++++	5544354210	4432210000	C. _____ P. 53000	Syphilis of central nervous system
101	++++	++++	5555555421	55543222210	C. _____ P. 55000	Syphilis of central nervous system
102	0	0	42211000000	22110000000	C. _____ P. 30000	Syphilis of central nervous system
103	++++	++++	55555554320	54332100000	C. _____ P. 54200	Syphilis of central nervous system
104	++++	++++	2234542l0	54321000000	C. _____ P. 53000	Syphilis of central nervous system
105	++++	++++	33335433321	55554222100	C. _____ P. 54000	Syphilis, meningovascular
106	+++	0	00001211100	33322110000	C. 31000 P. 55000	Syphilis, congenital
107	++++	0	00000000000	00000000000	C. _____ P. 20000	Syphilis, congenital ?
108	++++	++++	45555555210	5544321000	C. _____ P. 52000	Syphilis, congenital
109	++++	++++	33333554210	5543221000	C. 20000 P. 44000	Tabes dorsalis
110	+++	++++	1223442100	43221000000	C. _____ P. 50000	Tabes dorsalis
111	+++	++++	1111200000	00000000000	C. _____ P. 30000	Tabes dorsalis
112	++++	++++	22223321100	22211000000	C. _____ P. _____	Tabes dorsalis
113	+	++++	44544432l0	54433322ll	C. _____ P. 52000	Tabes dorsalis
114	0	++++	11112210000	32110000000	C. _____ P. 50000	Tabes dorsalis
115	0	++++	44322221100	33222110000	C. _____ P. 42000	Tabes dorsalis
116	++++	++++	54211221100	21100000000	C. _____ P. 20000	Tabes dorsalis
117	0	+++	44444333332	23321000000	C. _____ P. _____	Tabes dorsalis
118	0	0	00000000000	00000000000	C. _____ P. 20000	Thromboangeitis obliterans; Tabes dorsalis?
119	++	++++	55555555520	55554221100	C. _____ P. 55000	General paresis
120	++++	++++	45555555310	55554322210	C. _____ P. 54000	General paresis
121	++++	++++	55555555521	55443222l0	C. _____ P. 54000	General paresis
122	++++	++++	44001210000	23211000000	C. 10000 P. 43000	General paresis
123	+++	++++	00000000000	00000000000	C. _____ P. 40000	Diagnosis unknown
124	++++	++++	55555555432	55543210000	C. _____ P. 53220	Diagnosis unknown
125	No record	++++	55555555200	55555542ll	C. _____ P. 55000	Diagnosis unknown
126	0	0	33333210000	32211000000	C. _____ P. 40000	Multiple sclerosis
127	0	0	12223221100	42210000000	C. _____ P. 20000	Multiple sclerosis (advanced)

TABLE I—CONT'D

CASE	BLOOD WASS.	WASS. (SP. FL.)	LANGE	MASTIC	TAKATA-ARA	CONDITIONS
128	0	0	1223321000	1110000000	C. _____ P. 40000	Question of multiple sclerosis, syphilis, or brain tumor
129	0	0	0000000000	0000000000	C. _____ P. 30000	Paralysis agitans
130	No record	0	0000000000	0000000000	C. _____ P. 20000	Partial paralysis following anterior poliomyelitis
131	0	0	0000000000	0000000000	C. _____ P. 40000	Parkinson's syndrome
132	0	0	0001210000	2110000000	C. _____ P. 30000	Arterial hypertension
133	0	0	2222221000	1100000000	C. _____ P. 40000	Chronic sinusitis
134	0	0	2233421000	2221100000	C. _____ P. 20000	Organic disease of central nervous system
135	0	0	0011221000	1110000000	C. _____ P. 20000	Sciatic neuralgia
136	0	++++	4222220000	2110000000	C. _____ P. 30000	Pyelonephritis (history of syphilis)
137	0	0	0000111000	1111000000	C. _____ P. _____	Septicemia
138	0	0	0000000000	0000000000	C. _____ P. 30000	Symptomatic mental depression
139	0	0	2223333210	4443221100	C. 30000 P. 04000	Myelogenous leucemia
140	0	0	1222333344	5555555532	C. 33000 P. _____	Pyrexia
141	0	No record	0000122110	1111100000	C. _____ P. _____	Bilateral active pulmonary tuberculosis
142	0	No record	0001232100	1110000000	C. _____ P. _____	Cerebral hemorrhage
143	No record	0	0001222100	4432210000	C. 31000 P. _____	(Postmortem) chronic myelogenous leucemia
144	0	0	0000000000	0000000000	C. _____ P. 30000	Epilepsy
145	No record	0	0000000000	0000000000	C. _____ P. 20000	Laceration left eyelid
146	0	Ac.	0001111111	1245555555	C. 33321 P. _____	Pyrexia (undetermined origin)
147	0	0	5552222222	0122221000	C. 31000 P. 05000	Neoplastic lesion in paranasal sinus with intracranial extension
148	0	0	5552222211	1222210000	C. 32000 P. 15000	Neoplastic lesion in paranasal sinus with intracranial extension

Thus we have a simple test for examining spinal fluid specimens which appears to compare favorably with the older and more widely known Lange colloidal gold and mastic tests.

The percentage of positive reactions with each of the three tests in this series is: Mastic, 34 per cent; Lange, 36 per cent; and Takata-Ara, 38 per cent.

The chief advantage of the Takata-Ara test as observed is its simplicity. The tedious, time-consuming and often annoying procedures of preparing suitable colloidal gold solutions are dispensed with. The solutions of the reagents are very stable and need not be checked with a known positive spinal fluid every few days to see whether they are still good.

Compared with the mastic test it may be said to differentiate, with a fair

degree of consistency, between syphilis and the bacterial meningitides, a distinction which the mastic does not possess.

Its main disadvantage is that more spinal fluid is required to make the test.

The technic is given for performance of the Takata-Ara test with a method different from the original of recording results, which seems to be more easily grasped at a glance.

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THE KLINE SLIDE TEST AND THE WASSERMANN TEST*

By Hugh Jeter, M.D., A.S.C.P., and Madelene Norris, A.B.,
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THE interpretation of serologic tests, most particularly those which aid in the diagnosis of syphilis, is an undertaking which has become a very important and serious one. The biologic nonspecificity of our tests, and the recognition by both the laboratory expert and the clinician of occasional conflicting reports, have demanded certain facts in connection with interpretation. As a result of this demand, certain very essential procedures are beginning to be generally recognized as follows:

1. A standard complement-fixation or Wassermann test is important. In our experience, the Kolmer quantitative test is the best.

2. More than a single test should be used routinely. In the words of Stokes, "Refuse any longer to accept a report based on a single type of procedure, i.e., complement fixation only, or precipitation only. For ordinary practice, as well as for the expert, it is not too much to require both, and three or four tests of radically different types are often helpful."

3. In cases of disagreement between the laboratory report and the clinical findings, the tests should be repeated.

4. Proper training and experience are very essential for those who are to read and interpret serologic tests. The work of technicians should always be carefully supervised by an experienced serologist.

*From the Department of Clinical Pathology, University Hospital.
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We made a preliminary report on 2,222 comparative tests about eighteen months ago.

The purpose of this communication is to make a subsequent report of 4,405 sera tested in the University Hospital. Following the first report, Dr. Kline gave us very helpful minor suggestions, most of which are minor improvements in the technic of his slide test.

3,370 tests—77 per cent negative with both Kolmer and Kline.
687 tests—15 per cent positive with both Kolmer and Kline.

or

4,057 tests—92 per cent agreement.
167 tests—3.79 per cent were anticomplementary.

Of the 167 anticomplementary tests, 61 were positive with Kline.

Clinical results and repeated Kolmer tests showed over 90 per cent of the anticomplementary ones to have reliable Kline tests. In other words, the Kline was at least 90 per cent satisfactory, as far as we could determine in tests where the sera were anticomplementary with the Kolmer.

The clinical reports were, as nearly as possible, to ascertain, in agreement with the above reports. Approximately, 25 per cent of these were treated cases and negative with both tests.

Three hundred tests or 6.81 per cent were positive (3-plus or stronger) Kline and negative Kolmer. Of these, all but ten tests were clinically positive. The ten tests, not definitely positive, were cases of colored patients in the outpatient department, not definitely negative, but most of them suspiciously positive cases clinically.

Forty-four tests or 0.99 per cent were positive (44 – – – or stronger) with the Kolmer and negative with the Kline. Clinical investigation revealed 90 per cent of these cases to be positive, and the other 10 per cent were at least suspicious.

CONCLUSIONS

1. The results of 4,405 tests with both the Kolmer and Kline tests along with clinical comparison are given.

2. The Kline test is very satisfactory.

3. Tests for syphilis are more satisfactory if two or more different types of tests are used routinely and any discrepancies thoroughly studied.

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A QUANTITATIVE WASSERMANN TEST. PRELIMINARY REPORT*

By Maurice B. Wolff,† M.D., Chicago, Ill.

ONE point of paramount importance in the Wassermann reaction that needs stabilizing is a method of reading and reporting the tests so that the personal equation may be entirely removed.

With the present day method of reading it is a matter of experience only that makes various degrees of positive reactions, expressed 1, 2, 3, and 4-plus or 25, 50, 75 and 100 per cent positive, by various laboratories.

There is no accurate basis to these groups of readings and the fact is, they have no real meaning at all. What one expert reads as 50 per cent positive (or 2-plus), another expert may well read 25 (1-plus), or even 75 per cent (3-plus) positive. The reading is entirely qualitative and depends wholly upon the judgment and experience of the technician, who, at the most, compares the end results with controls from known positive and negative sera.

On account of this variation which cannot be helped, when the personal equation counts so much, it has rightly been suggested by many distinguished serologists and clinicians that the reporting of Wassermann tests be simplified and divided into three groups of qualitative meaning only. In other words to report the tests as positive, doubtful, and negative.

Outside of this one factor, the Wassermann test is well standardized, so that reports from different laboratories, on the same blood specimen, agree, except in the degree of positivity and this can never be corrected, unless the reporting is simplified or an accurate quantitative method of reading is accepted.

To this end, many serologists have worked on quantitative methods, using the human serum, the antigen and the complement as the basis with no results, so that with all the accepted stability of the Wassermann test, the reading and reporting remains the same. The layman or patient, having been given this method of reporting for so many years, in what he believes is an estimate or measurement of the status of his infection, is possibly the reason that the reporting has remained unchanged and is still expressed in quantitative values, although these values have no scientific basis and are therefore more or less erroneous.

My own experience with the Wassermann reaction dates back to its very first days, when Prof. Robert Zeit of Northwestern University Medical School translated Wassermann's original article, and we began doing the test in the medical school pathologic laboratory. A short time later I visited Wassermann's laboratory in Berlin, and ever since that time have been actively in-

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†Assisted by Miss Marian Wolter.

terested in serology in my own private laboratory and in connection with clinics, hospitals, and medical schools.

It is not the purpose of this article to discuss the comparative values of the Wassermann, the Kahn, or other methods, but it is felt that if an accurate quantitative Wassermann reading is accepted, it will completely stabilize the Wassermann and put it on top in a position by itself, far above any other modifications or agglutinations or precipitation known to the laboratory at the present writing.

At this time we wish to present a preliminary report of a quantitative reading of the Wassermann test, which has proved accurate and which in no way alters or interferes with the technic in its most minute part.

It will be necessary to discuss the test itself in order that the logic of the present idea will be understood.

The Wassermann test is primarily based upon the phenomenon of hemolysis according to Bordet-Gengou. A standard hemolysis is established consisting of a known amount of sheep red blood cells and titrated units of complement and amboceptor. In the presence of an antigen plus syphilitic blood serum, the complement is destroyed or inhibited of its activity, so that the hemolytic system is destroyed and no hemolysis occurs. This constitutes a positive Wassermann reaction. When there is no syphilis (anti-body or allergin) in the blood serum, the complement remains unchanged and active hemolysis takes place, thereby constituting a negative reaction.

When there is no hemolysis at all present, the Wassermann is read 100 per cent or 4-plus. When hemolysis is complete, the reaction is read negative. When some portion of the complement is destroyed, some degree of partial hemolysis exists and these constitute the varying degrees of positive reactions. Just what amount of hemolysis takes place is not figured by any method by the technician, but the tubes are read by simple macroscopic vision and at the most compared with known controls. Therefore by personal experience only, the technician quickly reads off the results by the appearance of the tubes and reports the results as some grade of positive or negative as they appear to be.

Now then, the actual basis of the Wassermann test is the standard hemolytic system and in this standard hemolytic system, the one factor that is always the same is the sheep cells. The complement and amboceptor are titrated into exact amounts called units, to completely hemolyze 0.5 c.c. of a 5 per cent suspension (in normal salt) of washed sheep cells in a specified length of time, usually thirty minutes at incubator (37° C.) temperature.

It is on this basic factor of a known amount of sheep cells that the present quantitative method is fixed and estimated.

If, when the Wassermann test is completed, there is no hemolysis, the sheep cells remain in the same condition and amount as when the test was started. If hemolysis is complete, the sheep cells are all destroyed and have disappeared. If hemolysis is partial in any degree, large or small, some quantity of the original sheep cells have been hemolyzed, while some remain intact as when the test was started.

The ratio or percentage of the cells destroyed by hemolysis and the cells remaining intact is compared to the sheep cells when the test was started.

gives an accurate method, by simple arithmetic of definitely measuring and expressing the exact degree of positive that exists in each individual test.

It is certainly a logical thought that the sheep cells can be measured or estimated as to just how many have been destroyed during the working of the test.

The general method or technic of the Wassermann test remains exactly the same, whether the original method, the Kolmer, or other routine is being used. The estimation for final reading is based entirely on the sheep cells and this is always a basic factor of a known amount in every method.

In our laboratory a 5 per cent suspension of washed sheep cells is used and this suspension is measured as accurately as is humanly possible. Of this suspension, 0.5 c.c. is used in the test. The complement and amboceptor are titrated into definite units, to work in a volume of 0.5 c.c. With the antigen, the human serum and the added normal salt solution, each tube at the end of the test contains a complete volume of 2.5 c.c. of which 0.5 c.c. is sheep cells.

The basis of the quantitative reading method is simply to count the number of sheep cells existing in a definite volume of blood before starting the test, to again count the sheep cells existing in the same volume of blood at the finish of the test and by the difference in the counts before and after, the percentage of loss of cells by hemolysis is estimated and the answer represents an exact percentage, which gives the degree or percentage positive of the Wassermann test under consideration.

Before applying the method, the sheep cells were counted under varying conditions to ascertain whether standing in the ice box, in the room, or in the incubator made any appreciable difference or diminution in the count, and it was proved that there was no change or difference in the length of time consumed by the entire Wassermann test. Of course, the counts were never equal down to a single cell, but it did not seem objectionable to allow a margin of variance the same as always occurs in any ordinary blood count and which makes no great difference in the end result. Such a condition exists in many laboratory procedures and gives the clinician a slight range, which up to a given point expresses a normal on either side. This clinical range exists in blood counts, blood chemistry, basal metabolism, gastric titrations, etc.

Different counting methods and different values were experimented with until it was decided as follows: an ordinary white blood counting pipette and blood counting chamber and Hayems solution completes the necessary equipment.

First, the sheep cells are freshly washed and a 5 per cent suspension in normal salt is made in the usual manner. Next, the suspension of sheep cells is further diluted with normal salt solution, to represent the total volume which is going to be present in the tube at the end of the test. In our work, we take 0.5 c.c. of the 5 per cent sheep cells and add 2.0 c.c. of normal salt solution, this representing the 2.5 c.c. volume of each individual Wassermann tube at the end of the second incubation. From this dilution, using the white pipette, draw up the diluted sheep cells to the 1 mark and pull up Hayems solution to the 11 mark, thus getting a dilution of 1 to 10. This is then

counted in a blood counting chamber in the usual manner of counting red blood cells and computed in the regular manner, except in estimating the dilution, which in this method is 1 to 10 and not 1 to 200 as in the blood count. This count will represent the number of sheep cells per 1 c.mm. in the volume being used in the test.

The results of the count run on an average of between 400,000 and 500,000 and sometimes a little over. This makes no difference as the count is made daily on freshly washed cells, just before doing the test, usually while the serums are being inactivated.

When the test is completed and ready for reading, the tubes are shaken and a red cell count repeated in the white pipette, at 1 to 10 dilution with Hayems solution. The loss in the number of sheep cells shows the number of cells hemolyzed. With the count before the test and the count after the test, the percentage of loss is simply determined. Only one standard count is done before the test and each individual tube seeming positive is counted separately and figured against the standard count.

For example, the sheep cells count before the test is 516,000, that is the standard count for the day.

At the completion of the test take positive tube number one and do a red cell count, which for example figures 345,000. This means that the missing cells have been hemolyzed.

516,000 | 345,000 | 668

3096

3540
3096

4440
4228

The test is 66.8 per cent positive.

Perhaps the next positive shows a count of 488,000.

516,000 | 448,000 | 945

4644

2360
2064

2960
2880

The test is 94.5 per cent positive.

In the negative tubes where hemolysis is complete or seems to be from macroscopic reading, the contents may be looked at under the microscope anyway. In every one of a large number of negative tests examined, there were never any red blood cells to be seen. When some red cells are found in what macroscopically appears to be a negative tube, a cell count is done and the percentage estimated. When only an occasional red cell is seen the margin is so close that the test is called negative, but several cases have been found from 5 to 20 per cent positive, which when reported as such proved valuable to the clinician in his diagnosis and treatment.

It is only necessary to do a count on the tubes that show some cloud or evidence of incomplete hemolysis and these suspected tubes, of course, are counted separately and estimated as described.

Since it is impossible to obtain two blood counts exactly alike down to a single cell, a reading of exactly 100 per cent positive will be very rare. So instead of reporting the actual arithmetical percentage, it is suggested to report the actual percentage in figures to the closest "5." In this way a 98.6 per cent would become 100 per cent, while either 97 per cent or 93 per cent would become 95 per cent positive.

A very large number of Wassermann tests have been read by this method through the courtesy of laboratory friends, doctors and hospital and school clinics, with great satisfaction to the technicians and especially to the doctors who have been receiving this type of report.

From my own experience, I find, that outside of unmistakable 4-plus positive reactions, my own tendency has been over high readings on the moderate positive tests as compared with the computed percentages. Also I find that some of the supposedly negatives have contained enough cells unchanged to give a mild positive percentage that has proved valuable to clinicians in treatment cases and in organic and latent syphilis.

Table I shows a number of positive tests with both styles of reading. A large number of these tests were clinically checked, through the courtesy of

TABLE I

POSITIVE PERCENTAGE QUANTITATIVE READINGS

NO.	CELL COUNT BEFORE TEST	CELL COUNT AFTER TEST	PER CENT POSITIVE	USUAL READING
1	532,000	40,000	7.5	2-plus
2		510,000	95.8	4-plus
3		415,000	76.1	4-plus
4	470,000	425,000	90.4	4-plus
5	518,000	502,000	96.9	4-plus
6		287,000	55.4	3-plus
7	555,000	520,000	93.9	4-plus
8		225,000	45.4	2-plus
9	469,000	465,000	97.0	4-plus
10		235,000	50.0	2-plus
11		60,000	13.4	negative
12		165,000	33.0	2-plus
13	599,000	158,000	26.0	2-plus
14	557,000	536,000	99.9	4-plus
15		80,000	10.5	negative
16		31,000	5.8	negative
17		165,000	30.7	1-plus
18	550,000	540,000	98.0	4-plus
19		472,000	85.8	4-plus
20		185,000	33.6	4-plus
21	462,000	458,000	99.1	4-plus
22		385,000	83.8	4-plus
23		235,000	50.0	3-plus
24		425,000	91.9	4-plus
25	518,000	220,000	42.6	2-plus
26		90,000	17.3	negative
27		122,000	23.5	2-plus
28		508,000	98.0	4-plus
29		450,000	84.9	4-plus
30	447,000	442,000	99.5	4-plus
31		305,000	68.2	2-plus

several doctors and clinics. It is useless to tabulate the negative tests, because in every one, there were no cells seen under the microscope. Only enough reports are tabulated to show that the idea is sound and that the reports are uniform.

CONCLUSIONS

1. Since the hemolysis of sheep cells is the basis of the Wassermann test and since this is always a definite known amount to which all other substances are titrated and standardized, it is the logical base for quantitative estimation.

2. The method is one of counting only and in no way interferes with any method of performing the test and requires only a few moments extra time.

3. If such a method is accepted it will universally stabilize the Wassermann test, so that reports from different laboratories will be equal and uniform in every case irrespective of the actual method being done.

4. It places the Wassermann report on an accurate basis which is readily interpreted by both the doctor and the patient.

5. It is exceptionally valuable in border-line cases, primary syphilis, latent and organic syphilis and especially in treated cases.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

PERNICIOUS ANEMIA: The Maintenance Dose of Potent Material, Beebe, R. T., and Lewis, G. E. Am. J. Med. Sc. 181: 796, 1931.

One hundred eight patients with pernicious anemia have been studied in an effort to determine the maintenance dose of liver or potent substitute. One hundred cases were placed in three groups and 8 composed a fourth special group, as follows:

Group 1. Sixty-four patients whose red blood cells continued to remain at a normal level when they took only relatively small amounts of effective substance. These patients were treated for the same amount of time as those in Group 2.

Group 2. Thirty-one patients who required large amounts of effective material to maintain a normal blood level.

Group 3. Five patients whose red cells remained about 20 per cent below normal even when very large amounts of potent liver material and iron were fed.

Group 4. Eight patients whose red cells and hemoglobin remained somewhat below normal when large amounts of liver extract were taken, but who obtained a normal blood level when iron was given with even a smaller amount of liver extract than formerly.

Observation of the latter group together with the study of a case in which chronic blood loss occurred, indicates that two sorts of disordered blood formation may occur in a patient with pernicious anemia.

The rôle played by infections and other complications in inhibiting the effect of potent material is mentioned.

The importance of arteriosclerosis in conditioning the response to liver therapy is suggested, because the average age of the patients and the incidence of arterial disease in the first three groups progressively increases; that is to say, a larger maintenance dose was required usually when arteriosclerosis was present.

The patients whose red blood cell level remained normal on the smaller amounts of liver, or potent substitute, showed on the average the greatest improvement in the function of their neuromuscular system. Thus, neuromuscular symptoms decreased less, as a rule, when arteriosclerosis or other complications were present. These symptoms only appeared or grew worse when the red blood cell level lapsed, or the patient took insufficient amounts of liver or potent substitute.

It is evident that some patients require a much larger maintenance dose of potent material than other patients. Optimal quantities of an effective substance to keep patients in as satisfactory a state of health as possible should be prescribed, rather than amounts that permit them to remain in a state where untoward events can develop easily. The daily maintenance dose is "*not some liver but enough liver for the given case.*"

HYPERTENSION: Carbohydrate Metabolism In, Wishnofsky, M., and Byron, C. S. Arch. Int. Med. 47: 790, 1931.

A dextrose tolerance test was performed on ten patients suffering from hypertension. The blood sugar and the respiratory quotient curves were studied simultaneously. In six of the ten cases the blood sugar during fasting was higher than normal. It was shown that all patients with hypertension have high blood-sugar curves. The respiratory quotient curves were normal in nine of the ten cases. This is adequate proof that there is no diminution in the secretion of insulin in hypertension, and that these patients are neither potentially nor mildly diabetic. It disproves the theory that the disturbance in carbohydrate metabolism in hypertension is caused by sclerosis of the blood vessels of the pancreas. In the case in

which the respiratory quotient curve was lower than normal, diabetes mellitus, independent of the hypertension, could be considered to exist.

The theory that hyperadrenalemia is the cause of the disturbance cannot be proved in the present state of knowledge.

The mechanism concerned in producing this disturbance in carbohydrate metabolism in hypertension is unknown and awaits further research for its elucidation.

BILIRUBIN: Serum and Plasma, Jacobi, M., Finkelstein, R., and Kurlen, R. Arch. Int. Med. **47**: 759, 1931.

The results of simultaneous quantitative determinations of bilirubin by the Thannhauser and Anderson modification of the van den Bergh technic in 100 consecutive and unselected cases are reported, and the results are analyzed as to the degree of agreement or disagreement.

The readings are shown to correspond in a very high degree, and they indicate that plasma instead of serum may be used with equal accuracy in determinations of bilirubin.

The results confirm the statements of McNee and Keefer. They are directly opposed to the figures reported by Shay and Schloss from eleven observations with wide discrepancies in the readings.

An increase in the length of time allowed for coupling up to one hour apparently plays no part in the slight quantitative differences noted.

BILIRUBIN: Detection of in Urine, Hunter, G. Canadian M. A. J. **23**: 823, 1930.

To 5 c.c. of urine in a 15 c.c. centrifuge tube add 2 c.c. of 10 per cent barium chloride, mix, and centrifuge. Pour off the supernatant fluid and wash the precipitate with a few centimeters of water, again centrifuge, and discard the supernatant fluid.

Add 0.5 c.c. of diazo reagent and stir the precipitate with a glass rod. Add 2 c.c. of 96 per cent alcohol and 0.3 c.c. of 6 per cent primary sodium phosphate.

If bilirubin is present the same color reaction occurs as with icteric serum and diazo reagent.

Alkaline urines must be made weakly acid with acetic acid.

ADRENALIN: Detection of in Blood, Viole, G. Bull. Soc. ital. Biol. Sper. **5**: 1163, 1930.

To 1 c.c. of blood add 1 c.c. of a saturated aqueous solution of bichloride of mercury acidified with a trace of hydrochloric acid (pH 6).

Filter and add to the filtrate a "few" drops of a saturated solution of sulphanilic acid and a saturated solution of potassium iodide.

In the presence of adrenalin a rose color develops. The reaction is said to be highly specific and sensitive to a dilution of 1:30 million.

SPRUE: Differentiation From Pernicious Anemia by Morphology of Neutrophilic Leucocyte, Tyner, J. Clifton Springs Med. Bull. **17**: 86, 1931.

The typical neutrophilic leucocyte of pernicious anemia has been described. It is usually larger than normal. Most of the nuclei, as the Arneth index of 32.45 indicates, are found to consist of 3-4-5 segments and not infrequently of 6 and 7 segments. The individual segments are small and show some, but not marked, vacuolization and most noticeably are connected to each other by fine delicate strands. There is nothing remarkable about its cytoplasm or the cytoplasmic granules.

The neutrophilic leucocyte of tropical sprue, while not typical of the disease alone is nevertheless quite constant and usually resembles those seen in any mildly toxic condition. It is about normal in size. The approximately normal Arneth index of 62.61 determines that the nuclei of most of these cells are of the 2-3-4 segment type as in a normal or mildly

regenerative state. The individual segments are quite large, often noticeably vacuolated, of the thick heavy bar type, and often intertwined among each other. Knoblike projections at the ends of the segments or in their bellies are frequent. Connections between the nuclear segments are broad. The cytoplasmic granulations are coarse and stain deeper than normal, as does the cytoplasm itself.

The more important points to be noted are the large size of the neutrophilic leucocytes in pernicious anemia and the larger number of small nuclear segments with delicate stranding, and little vacuolization; contrasted to the normal sized cell in sprue containing fewer but larger nuclear segments, with a more toxic vacuolated appearance and often connected by broad bands. Frequent 5-6-7 segment cells speak for pernicious anemia and against sprue while one segment cells denote sprue rather than pernicious anemia, which is simply another way of stating the Arneth indices already obtained. The cytoplasmic granules are coarse and deeply staining in sprue; those of pernicious anemia are not noteworthy.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*The Long Trek**

WHAT doctor, seated in his consultation chamber, listening patiently or half listening, to a wordy description of the minutiae and vicissitudes of his patient's headache or hyperperistalsis does not occasionally long to chuck the whole show and go where patients are unknown and the only abdominal pain requiring relief is the occasional pang of hunger. Few of us are so fortunate as to be able to gratify these whims. Doctor Sutton has, however, made it possible for us to procure a vicarious enjoyment of such a trip at home in the evening, as we travel with him through the heart of Africa and on around the world in his delightful chronicle of the long trek.

It is no more than a diary of their progress from day to day through remote lands but with thrills aplenty, in the search for big game and with a descriptive charm which carries the reader along as though he were a member of the party. There is an abundance of illustrations, all photographs taken during the trip. The final chapter presents necessary details for those who might wish to make similar voyages.

There is a lesson to be gained from this volume. Here is an extremely busy practitioner of medicine who does find the time to do that which all of us would like to do but feel that we cannot for want of time. Unless misfortune meets him on one of his journeyings he will live longer than the rest of us who keep our faces too close to the grindstone, and in either event he will have gotten more out of life. If the reading of his volume does no more than to show the rest of us what a busy doctor can do outside of medicine and inspire us to emulate him in one way or another, it is well worth the reading.

The Story of San Michele†

IT IS enough out of the ordinary for an autobiographical treatise by a physician to become a best seller, to try to analyze the wherefores. Why have Dr. Munthe's animadversions on his past gone through more than thirty printings in this country alone since they first appeared in July, 1929? What element in the story has caught the popular fancy? Or has it been several elements, each with its appeal to a different type of reader? The reviewer upon closing the cover finds no single explanation. Most persons enjoy hearing or reading of the mistakes and weaknesses of members of the medical profession. The author does not hesitate to present them when they are requisite to complete his story. There is enough

*The Long Trek. Around the World with Camera and Rifle. By Richard L. Sutton, M.D., Sc.D., LL.D., F.R.S.(Edin.). Fellow of the Royal Geographical Society; Member of the French Geographical Society; Professor of Dermatology, University of Kansas. With more than 200 illustrations from photographs made by the author, and by Richard L. Sutton, Jr., A.M., B.Sc., M.D., Fellow of the Royal Geographical Society. Cloth, pages 347. St. Louis. The C. V. Mosby Company, 1930.

†The Story of San Michele. By Axel Munthe. With New Preface by the Author for the American Edition. Cloth, pages 380. New York. E. P. Dutton & Co., Inc. 1930.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

of the neurotic in each of us to allow us some pleasure in reading of the foolishness of other neurotic patients. There is always the gossipy thrill of "getting the inside dope" on your neighbor even if his name remains anonymous.

But it is above all, I believe, the charming manner in which the story is told which has established its popularity.

Every physician should know the story of San Michele if for no other reason than that he should know what his patient, who has read it, is talking about.

There are bits of medical history that have never been told before. Such was Pasteur's ward experience with rabies. There are bits of medical history that have never been told better. Such was the story of the cholera in Naples. There are bits of anecdotes which are recounted among doctors but less often to the public. Such was the experience of the Russian general. There are bits of romance which one cannot but feel have been slightly embellished to improve the story. Such was the tale of the English lady and her child.

Scattered through the volume one runs expectedly upon little gems of philosophy. We cannot refrain from reproducing Dr. Munthe's respects to the telephone:

"Telephone, that deadly weapon in the hands of idle women, not yet started on its nerve racking campaign against every hour of well-earned rest." To this sentiment every practicing physician will add his amen.

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*Clinical Allergy**

THIS is a most thorough review of what is definitely known today concerning clinical allergy and experimental anaphylaxis as it touches upon clinical allergy, into which the author has incorporated his own wide experiences, theories, and conclusions. At no place in the volume does he allow these latter to receive undue prominence or to overshadow the equally constructive work of other investigators.

The major portion of the volume is devoted to inhalant allergy and this is as it should be not only because the author's major interest has been in this field but also because it is that portion of the allergic problem which has been most thoroughly studied.

Most questions such as the existence of true bacterial allergy are not merely glossed over but are analyzed pro and contra. This monograph is altogether different from any of the several other books which have appeared within recent years on allergy in that while the others possibly with one exception, deal with special phases of the allergic diseases, the volume under review discusses the problem in its entirety. Possibly the division of alimentary allergy might have received more detailed consideration but on the other hand we must recognize that our knowledge on this phase of the problem has not progressed as far as it has with inhalant allergy.

This is a volume which will at once find its way to the bookshelves of all clinical allergists and one which should be read by the clinician not primarily interested in allergy so that he may acquaint himself with the vast work which has been done in this field in order to make better use of this knowledge in the treatment of his frank and borderline allergic patients.

Alimentary Anaphylaxis†

SEVERAL years ago there appeared a little paper-bound booklet in French written by Laroche, Richet and Saint Girons entitled *L'Anaphylaxie Alimentaire*. This work though small and scarcely read at all in this country was of utmost developmental and

*Clinical Allergy. Particularly Asthma and Hay Fever. Mechanism and Treatment. By Francis M. Rackemann. Physician to the Massachusetts General Hospital Instructor in Medicine, Harvard Medical School, Boston, Mass. Cloth, pages 617. New York. The Macmillan Company. 1931.

†Alimentary Anaphylaxis. (Gastro-intestinal Food Allergy.) By Guy Laroche, Charles Richet Fils and Francois Saint-Girons, Paris, France. Foreword by Professor Charles Richet, of the Faculty of Medicine of Paris. Member of the Institute and of the Academy of Medicine. Translated by Mildred P. Rowe and Albert H. Rowe. Preface by Albert H. Rowe. Cloth, pages 139. University of California Press, Berkeley, California. 1930.

historical importance in allergy in that it represented the first serious systematic consideration of food allergy in France. In 1919 food allergy was new neither in France nor in the United States. In both countries the condition was known and was receiving increasing discussion. In this country skin tests were being applied and positive reactions were being accepted as evidence of food allergy. Negative skin reactions were interpreted as indicating an absence of food allergy. Emphasis was placed upon these skin tests. In France on the other hand little attention was paid to cutaneous testing while much was given to study of the patient's dietary in a search for offending foods. Today the French are coming to use the sensitization test much more widely, while in this country we are realizing that skin testing is but half of the story and that we must study the patient's diet much more thoroughly in a search for allergenic foods which give negative skin reactions.

So allergists in each country have profited by the experience of their confreres in the other, and all have discovered that for best results both systems must be applied. It is altogether fitting that Doctor Rowe who was one of the earliest proponents of the elimination diet and the food diary in this country, with the able assistance of Mrs. Rowe, should make available an English translation of this epochal French work.

Many of the case histories presented by the French authors are of great interest and outstanding among them are their accounts of cases of major anaphylaxis, even with death, following the ingestion of allergenic food. Another outstanding contribution which appears in the pages is their demonstration that guinea pigs can be sensitized to foods by alimentary ingestion. This anticipated the more recent work which has conclusively demonstrated that whole proteins may pass undigested through the intestinal mucosa into the circulation.

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*Heart Disease**

THIS is the most comprehensive clinical work on the heart that has appeared within recent years. The first three hundred pages are devoted to methods of examination including a comprehensive discussion of the history, physical examination, sphygmomanometer, roentgenology and electrocardiography.

Part two discusses the incidence, causes and types of heart disease and this is most interesting. The author presents an original classification in that he starts with the conditions found at birth and presents in succession the cardiac diseases that develop at the different ages of life. First, of course, come congenital cardiovascular defects and this is followed by rheumatic heart disease, bacterial endocarditis, cardiovascular syphilis, the specific infections such as diphtheria and tuberculosis, thyroid disease, hypertensive heart disease and coronary disease.

The third section is devoted to more comprehensive study of the abnormalities of the myocardium, pericardium and endocardium, the vascular system, and the fourth section deals with functional disorders such as congestive failure and the arrhythmias. The sequence is beautifully worked out.

All parts of the volume make excellent reading but the second section above described is of especial value as a reference work. The bibliography comprising as it does nearly two hundred pages indicates the vast amount of research indulged in by the author.

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*Heart Disease. By Paul Dudley White, M.D., Instructor in Medicine, Harvard Medical School; Physician, Massachusetts General Hospital, Boston. Cloth, pages 931. New York. The Macmillan Company, 1931.

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EDITORIALS

The Prophylaxis of Tetanus

THAT the routine prophylactic administration of tetanus antitoxin has been in large measure effective in the prevention of this disease, there is ample statistical evidence to demonstrate, but, if the matter were closely investigated and an extensive clinical survey conducted, there is some reason to believe that the present confident reliance upon the procedure as routinely conducted is not altogether warranted.

Perhaps the majority, if questioned, would probably give 1500 units as an ample and satisfactory dose of tetanus antitoxin. While it is true that it is impossible to state with any degree of certitude which of the patients to whom such a prophylactic dose has been given would have developed the disease if the antitoxin had not been administered, the marked decline in the incidence of tetanus following the institution of this prophylactic measure proves beyond question its very definite and consistent efficiency.

That it cannot be applied in routine fashion with an unquestioning confidence, however, there is ample, though not always reported, clinical experience to demonstrate.

It appears timely, therefore, to consider whether the prophylactic administration and dosage of tetanus antitoxin should not be removed from the realm of the routine and the empirical and considered in the light of the individual case and the particular features associated with it.

The question has been touched upon before but well deserves renewed comment.

There can be few large hospitals whose records will not show cases in which tetanus has developed despite the administration of the usual prophylactic dose.

This is not incomprehensible if the factors determining the relation between bacterial invasion and the development of disease are even cursorily reviewed.

The invasion of the body tissues by bacteria is, of course, an essential preliminary to the subsequent development of infection and disease. But the mere fact that bacterial invasion has occurred does not necessarily nor inevitably predicate that disease will follow.

There are various factors which must be considered and which definitely determine the results to follow, among them the virulence of the invading organisms, or their inherent aggressiveness and ability to attack the tissues; the degree of invasion, that is, the numerical proportion in which the bacteria have been planted in the tissues; and, as opposed to these factors, the ability of the patient to resist the bacterial attack.

It is apparent, therefore, that by varying the relation of these factors one also varies the likelihood and the probability with which disease will follow bacterial invasion; and, as these factors and this probability vary, so must measures to combat them vary also.

In other words, while the prophylactic administration of 1500 units of tetanus antitoxin may and usually does suffice where the injury is relatively slight and tetanus bacilli are present in but small numbers; that it will not infallibly suffice when the injury is grave or extensive and complicated by massive bacterial invasion clinical experience supplies enough examples.

It is certainly logical to suppose that a minor injury from a relatively clean instrument is less likely to be followed by tetanus than an equally minor injury inflicted by an instrument of a character much more likely to be infected with tetanus spores, as a manure fork, for example. It is equally logical to infer that not only the character but also the extent of the injury, as well as the circumstances under which it takes place, will all have an important influence upon the incidence of tetanus.

Even fairly grave tissue damage sustained under relatively cleanly circumstances is less likely to be the source of tetanus than an injury of similar or lesser proportions into which, however, the refuse of the stable or the street have been well and thoroughly implanted.

The experiences of the World War amply demonstrate the logic of such concepts and, indeed, of the point at issue; for an order, not always carried out, issued by the French provided for the repetition of prophylactic doses of

tetanus antitoxin, thus recognizing the dangers of unlimited reliance upon a single dose of purely arbitrary proportions.

It would seem advisable, therefore, to regulate the prophylaxis of tetanus by some logical principle based upon the particular circumstances. Where the injury is of relatively minor character and the probability of its infection with tetanus spores relatively slight, the usual dose of 1500 units may be regarded as sufficient.

Where the injury is grave, extensive, or of a character, or sustained under circumstances rendering its infection with tetanus spores extremely probable, then, just as the surgeon regulates the extent and character of drainage by the extent and character of the wound, so should the prophylaxis of tetanus be regulated by similar considerations.

In such cases the prophylactic dose may well be doubled to 3000 units and wisely repeated, either in the same or one-half this amount not more than five days later. In fact, as the efficiency of the antitoxin depends upon its continued presence in the body fluids, it may well be given, perhaps, partly subcutaneously and partly intramuscularly in order, by the slow absorption in the latter case, to provide a depot from which it may be continuously absorbed over some period of time.

The occurrence of severe anaphylactic reactions is guarded against by keeping the interval between doses not more than five days.

It is quite possible that this procedure may be unnecessary in some of the cases to which it is applied. It is also true that tetanus which would otherwise occur will be thereby prevented in others.

Considering the unsatisfactory results in the treatment of developed tetanus and the fact that its prophylaxis cannot always be safely anticipated by the usual 1500 unit dose of antitoxin, it is well worth considering if this method should not be applied in conformity with logical principles rather than based merely upon an empirical assumption.

—*R. A. K.*

Jamaica Ginger Paralysis

THE addition of various substances to alcohol and alcoholic preparations in order to prevent their use as beverages has added to the clinical problems confronting the physician a number of often very puzzling syndromes, among them the so-called "Jake paralysis" which has been shown to be due to the ingestion of Jamaica ginger containing triorthocresyl phosphate.[1]

Goodale and Humphreys[2] recently report upon extensive histologic studies in three fatal cases of the anterior horn cells of the spinal cord and of the nerves supplying the paralyzed tissues (radial, anterior tibial, external popliteal, and sciatic).

In one case they were able to confirm the presence of an acute perineuritis of the cauda equina as reported by Jeter.[3]

In all three cases studied there was myelin sheath and axis cylinder de-

generation of the radial, sciatic, external popliteal, anterior tibial, and posterior tibial nerves.

These observations are of definite value as indicating the prognosis of the condition and accord with observations in nonfatal cases showing a persistence of the loss of foot motions but improvement in the wrist and finger motion.

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—*R. A. K.*

Correction

In the article on Rose Bengal Tests for Liver Function, by Delprat and Stowe, Volume 16, page 924, June, 1931, the last paragraph, "Technic of Test," the first sentence reading: "Irrespective of body weight approximately ten c.c. of a ten per cent dye solution is injected in an arm vein" should read "Irrespective of body weight approximately ten c.c. of a one per cent dye solution, etc."